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THE CHOLINE CONTENT OF FEEDS [1]

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(Received for publication August 3, 1942)

In view of the recent discovery of the relationship of choline and manganese to the prevention of perosis in turkey poults and chicks, the determination of the choline content of the common feeds is of importance. Jacobi, Bauman and Meek ('41) have published a method for the determination of choline in rat tissue, and state that it has been applied to a mixed ration fed to rats. Data are available concerning the choline content of several animal tissues and of some pure compounds, especially as they are related to lipotropic action in the liver (Fletcher, Best and Solandt, '35). The authors have been unable to find any published reports of the choline content of common feeds.

The method of Jacobi, Bauman and Meek ('41), slightly modified to be more applicable to the analysis of feeds, was used to determine the relative choline contents of a number of common poultry feeds. The method consists essentially of the extraction of the choline-containing substances from the feed with a mixture of equal parts of ethyl alcohol and ether, and hydrolysis of the extract with barium hydroxide. The liberated choline is estimated colorimetrically as an acetone solution of the reineicke salt. Jacobi, Bauman and Meek ('41) found the maximum absorption of light by choline reineckate to be at 525 mμ and the light absorption to be a linear function of concentration over the ranges studied, 0.5 to 9 mg. A Coleman spectrophotometer, type 10-S, was used to make the color comparison.

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The values for choline content of feeds given in table 1 are indicative of the results obtained by this method. It is readily seen that the cereal grains are poor sources of choline in the ration, the common grains containing only 0.020 to 0.050%. Animal products such as liver meal and fish meal are good sources of choline. Soybean oil meal and meat meal are intermediate in choline content.

TABLE 1

The choline content of feeds.

(Expressed as per cent choline)

SAMPLE	NO. SAMPLES	RANGE	AVERAGE
Corn	2	0.020, 0.021	0.021
Wheat	2	0.041, 0.022	0.032
Oats	2	0.056, 0.029	0.042
Wheat bran	2	0.050, 0.040	0.045
Dehydrated alfalfa	2	0.050, 0.077	0.064
Pea meal	1		0.065
Skim milk	2	0.047, 0.035	0.041
Dried whey	1		0.070
Casein	1		0.021
Soybean oil meal	2	0.156, 0.075	0.116
Liver meal	2	0.788, 0.550	0.669
Meat scrap	2	0.094, 0.075	0.085
Herring meal	2	0.280, 0.352	0.316
Salmon meal	1		0.250
Pilchard meal	1		0.205
Fish meal	1		0.289
Fish meal (chiefly dogfish)			0.340
Av. of all fish meals			0.286

Several mixed rations have been analyzed for choline by this method. Since the choline contents of the constituents were also known, that for the ration could be calculated. Table 2 shows the results obtained when the choline contents yielded by these methods are compared.

TABLE 2

Choline content of mixed rations.

	CALCULATED	BY ANALYSIS
	%	%
Ration A	0.022	0.022
Ration A + choline chloride	0.222	0.245
Ration B	0.030	0.033
Ration C	0.029	0.031

Recently Engel ('42) compared a number of methods for extracting choline compounds from feeds. He concluded that methyl alcohol was the most satisfactory solvent. Using methyl alcohol instead of ethanol-ether for extracting the feed samples the results given in table 3 were obtained. In all cases the methyl alcohol gave higher values.

TABLE 3

Comparison of the choline content of feeds as determined on the ethanol-ether and methyl alcohol extracts.

SAMPLE	CHOLINE CONTENT (PER CENT)	
	EtOH-ether ext.	MeOH ext.
Oats	0.06	0.11
Corn	0.02	0.06
Soybean oil meal	0.16	0.28
Soybean oil meal	0.08	0.34
Pea meal	0.07	0.16
Meat meal	0.09	0.16
Liver meal	0.79	1.10
Liver meal	0.55	0.92
Pilchard meal	0.21	0.38
Herring meal	0.35	0.44
Casein	0.02	0.03
Dehydrated alfalfa	0.05	0.10

Data have been obtained from several different feeding experiments designed to determine the perosis preventing properties of rations compounded from the analyzed ingredients. A negative coefficient of correlation of -0.785 existed between the choline content, as determined by analysis, using the ethanol-ether extraction, and the incidence of perosis in turkey poults. This correlation was highly significant. The results obtained in one experiment are shown in table 4. Choline was determined on both the ethanol-ether and the methyl alcohol extracts in this case. The choline content is given in per cent and the incidence of perosis is expressed as the perosis score (Evans, Robertson, Rhian and Wilhelm, '42). All rations contained added manganese. The basal diet referred to is the one designed by Jukes ('40) for the study of perosis.

Although the general correlation between choline content of the diet, using the ethanol-ether extraction, and incidence of perosis was good, some disturbing discrepancies in this relationship were evident. For example (table 4), although the diets containing soybean oil meal and pea meal contained the same amount of choline, there was a much greater incidence of perosis on the diet containing pea meal. Diets containing added choline hydrochloride did not fit into this relationship as well as would have been expected and were not included in these calculations.

TABLE 4

The relationship of choline in the diet to perosis in poults.

DIET	CHOLINE		PEROSIS
	Ethanol-ether ext.	Methyl alcohol ext.	
	%	%	*(score)*
Turkey starter	0.05	0.18	0.80
Basal + soybean oil meal	0.03	0.15	1.80
Basal + pea meal	0.03	0.11	3.00
Basal + fish meal	0.05	0.14	2.08
Basal + liver meal	0.08	0.25	0.57
Basal + 0.2% choline	0.28	0.29	0.57

The choline content determined on the methyl alcohol extract appears to be more closely related to the prevention of perosis than was the case when the ethanol-ether extraction method was used. (Coefficient of correlation —0.884). The discrepancies previously noted are cleared up when methanol extraction was used. Soybean oil meal contained more choline than pea meal, which appears to explain the difference in perosis-preventing activity. The diet containing added choline hydrochloride conformed to the relationship between perosis-preventing activity and choline content when the determination of the latter involved methyl alcohol extraction.

SUMMARY

The choline content of a number of feeds has been determined.

Cereals are poor sources of choline, and animal products are good sources.

There was a significant negative correlation between the choline content of the diet and the incidence of perosis in turkey poults.

LITERATURE CITED

ENGEL, R. W. 1942 Modified methods for the chemical and biological determination of choline. J. Biol. Chem., vol. 144, p. 701.

EVANS, R. J., E. I. ROBERTSON, M. RHIAN AND L. A. WILHELM 1942 The development of perosis in turkey poults and its prevention. Poultry Science, vol. 21, p. 422.

FLETCHER, J. P., C. H. BEST AND O. M. SOLANDT 1935 The distribution of choline. Biochem. J., vol. 29, p. 2278.

JACOBI, H. P., C. A. BAUMAN AND W. J. MEEK 1941 The choline content of rats on various choline-free diets. J. Biol. Chem., vol. 138, p. 571.

JUKES, T. H. 1940 Effect of choline and other supplements on perosis. J. Nutrition, vol. 20, p. 445.

THIAMINE, RIBOFLAVIN, PYRIDOXINE AND PANTOTHENATE DEFICIENCIES AS AFFECTING THE BODY COMPOSITION OF THE ALBINO RAT [1]

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(Received for publication July 13, 1942)

In a previous report (Voris, Black, Swift and French, '42) it was shown that thiamine, riboflavin, pyridoxine and pantothenate each affected the appetite of the rat in a characteristic manner, and that riboflavin, pyridoxine, and pantothenate promoted growth independent of food intake. In male rats, thiamine had no definite effect on growth unrelated to its appetite effect, but a favorable influence of thiamine on growth was observed in female rats.

These results emphasized the importance of differentiating between the appetite and the growth effects of dietary factors, especially when gain in body weight is used as the sole criterion in quantitative assays. The quantity of food eaten is dependent on the appetite of the animal and the palatability of the food at his disposal, while growth is dependent, not only on the quantity of food eaten, but also on the nutritive efficiency of the food.

Furthermore, after the cause of the increase in body weight has been established, it is important for the fundamental understanding of nutritional function to ascertain whether the composition of this increase is normal to the species investigated.

Thus, in order to characterize the growth differences observed in the investigation noted above, the bodies of the rats

[1] Authorized for publication on July 10, 1942, as paper no. 1116 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

were analyzed and the gains expressed in terms of water, protein, fat and residual matter. The results of these analyses are presented in this report.

The growth differences were results of moderate deficiencies of the vitamins tested and do not necessarily represent the pathological effects of extreme deficiencies.

EXPERIMENTAL

A detailed description of the diets and of the experimental procedure was given in the previous report (Voris et al., '42). Paired litter-mate rats were used for comparing a complete diet adequately fortified with the respective vitamins (ration A) with diets of the same basic composition but deficient in thiamine (ration B), riboflavin (ration C), pyridoxine (ration D), or pantothenate (ration E). Also, ration A fortified with liver extract (ration A_1) was used to ascertain the completeness of ration A.

After 10 weeks of controlled feeding on the experimental diets, the rats were killed with illuminating gas and the contents of the alimentary tracts were removed.

The empty bodies were then minced, and dried to constant weight over sulfuric acid in vacuum desiccators. The loss in weight represented the water content of the rat bodies at the end of the experiment.

The dried bodies were extracted with anhydrous ether for 48 hours in Soxhlet extractors to obtain the fat.

The residues from the ether extraction were exposed to atmospheric conditions until constant weight was established, and were then pulverized in a micro hammer mill. The nitrogen in the fat-free, dry bodies was determined by the Kjeldahl procedure, and protein was estimated as $N \times 6.0$ (Addis et al., '36).

The difference between the total empty weight of the body and the sum of the weights of water, protein and fat was designated residual matter. This fraction — the non-protein, non-fatty, dry substance of the bodies — was mainly mineral matter.

The gain of each constituent during the experimental period was determined by the difference between the composition of the rats at the end of the experiment and of other animals selected to represent the test rats at the beginning of the experimental feeding.

RESULTS

The analysis of the body gains is recorded in table 1. The results in this table are described statistically in terms of the standard errors of the means but, since the individual results were paired variates, the significance of differences was determined by the use of Love's ('24) modification of Student's table.

The water, protein and residual gains are presented in relation to the fat-free gain. Also, water and protein are presented in relation to each other. The results expressed in this way segregate the highly variable fat gains and serve to indicate any irregularities in the water-protein relationships attributable to a vitamin deficiency.

The absolute gains indicate the quantitative differences resulting from the differences in vitamin intake. In all comparisons, excepting that between rations A and A_1 in the last two lines of table 1, the gains resulted from identical food intakes of diets differing only with respect to a particular vitamin. In the exception noted the food intakes differed only slightly (Voris et al., '42).

In the following presentation of the results the terms "high" and "low" are used to designate the specific vitamin status of the rats which received the complete ration A and those which received the rations deficient in a particular vitamin.

Low thiamine. Both males and females on the low-thiamine ration B gained slightly less fat, on the average, than did their high-thiamine pair-mates. The difference was not statistically significant for either sex considered separately but, considered together, the odds were 30 to 1 against the difference occurring by chance alone.

TABLE 1

The average composition of body gains of rats as affected by deficiencies of thiamine, riboflavin, pyridoxine and pantothenate.[1]

RATION	VITAMIN INTAKE	NO. OF RATS AND SEX	DISTRIBUTION OF GAIN				COMPOSITION OF GAIN				WATER / PROTEIN
			Fat	Water	Protein	Residual	Fat [2]	Water [2]	Protein [2]	Residual [2]	
	γ/day		*gm.*	*gm.*	*gm.*	*gm.*	%	%	%	%	
					Thiamine						
B: low thiamine	10	5 ♂	11.9 ± 2.3	91.6 ± 5.0	32.7 ± 1.6	8.5 ± 0.3	8.2 ± 1.3	69.0 ± 0.2	24.6 ± 0.1	6.4	2.80
A: complete	81	5 ♂	15.2 ± 2.2	89.2 ± 4.7	31.8 ± 1.4	8.0 ± 0.2	10.5 ± 1.3	69.1 ± 0.3	24.7 ± 0.2	6.2	2.81
B: low thiamine	10	5 ♀	14.6 ± 4.5	64.7 ± 2.1	23.5 ± 0.6	7.0 ± 0.3	13.3 ± 3.6	68.0 ± 0.3	24.7 ± 0.2	7.3	2.75
A: complete	80	5 ♀	17.0 ± 3.1	71.4 ± 3.0	25.8 ± 0.9	6.7 ± 0.2	14.1 ± 2.3	68.6 ± 0.4	24.8 ± 0.3	6.6	2.77
					Riboflavin						
C: low riboflavin	10	5 ♂	25.5 ± 3.9	102.0 ± 3.9	35.6 ± 1.6	10.6 ± 0.4	14.7 ± 1.1	68.8 ± 0.3	24.0 ± 0.4	7.2	2.87
A: complete	103	5 ♂	39.5 ± 5.6	108.6 ± 2.6	37.2 ± 1.4	11.5 ± 0.4	20.1 ± 2.1	69.0 ± 0.1	23.6 ± 0.2	7.4	2.92
C: low riboflavin	9	5 ♀	23.1 ± 4.3	71.9 ± 0.8	25.5 ± 0.8	7.5 ± 0.5	18.0 ± 2.3	68.5 ± 0.5	24.3 ± 0.3	7.2	2.82
A: complete	5	5 ♀	30.8 ± 3.5	71.0 ± 1.4	25.9 ± 0.7	7.7 ± 0.2	22.7 ± 1.8	67.9 ± 0.1	24.8 ± 0.2	7.3	2.74
					Pyridoxine						
D: low pyridoxine	4.2	5 ♂	23.3 ± 4.8	83.7 ± 1.5	29.7 ± 0.7	9.0 ± 0.5	16.0 ± 2.6	68.4 ± 0.4	24.3 ± 0.2	7.3	2.82
A: complete	42	5 ♂	25.1 ± 2.8	93.5 ± 5.8	32.9 ± 1.9	8.9 ± 0.7	15.6 ± 0.9	69.1 ± 0.4	24.3 ± 0.4	6.6	2.84
D: low pyridoxine	3.6	5 ♀	18.2 ± 3.9	49.4 ± 4.8	18.6 ± 1.5	6.8 ± 0.6	19.6 ± 2.8	65.9 ± 0.6	24.9 ± 0.5	9.2	2.66
A: complete	36	5 ♀	16.4 ± 5.2	54.2 ± 5.7	20.7 ± 1.9	6.0 ± 0.2	16.9 ± 4.2	67.0 ± 0.8	25.6 ± 0.2	7.4	2.62
					Pantothenate						
E: low pantothenate	19	4 ♂	17.9 ± 5.4	82.7 ± 7.6	30.4 ± 2.9	8.5 ± 0.7	12.8 ± 2.6	68.0 ± 0.2	25.0 ± 0.2	7.0	2.72
A: complete	172	4 ♂	24.2 ± 5.4	92.7 ± 8.0	32.8 ± 2.9	9.1 ± 0.6	15.2 ± 2.6	68.9 ± 0.7	24.4 ± 0.5	6.7	2.83
E: low pantothenate	18	5 ♀	13.6 ± 2.5	65.6 ± 4.4	23.3 ± 1.7	7.7 ± 0.9	12.3 ± 1.9	67.9 ± 0.5	24.1 ± 0.2	8.0	2.82
A: complete	159	5 ♀	22.7 ± 5.9	70.5 ± 3.7	25.6 ± 1.4	7.4 ± 0.6	18.0 ± 3.5	68.1 ± 0.5	24.7 ± 0.1	7.2	2.75
					Liver Extract						
A: complete		2 ♀	40.7 ± 2.9	84.4 ± 0.1	30.4 ± 0.3	9.4 ± 1.1	24.7 ± 1.6	67.2 ± 0.5	24.2 ± 0.0	7.5	2.78
A_1: A + liver extract		2 ♀	38.2 ± 1.4	84.2 ± 2.8	30.8 ± 0.3	9.2 ± 0.7	23.5 ± 1.2	67.8 ± 0.1	24.8 ± 0.5	7.4	2.73
A: ad libitum		5 ♀	35.5 ± 4.2	90.0 ± 2.9	32.2 ± 1.0	9.5 ± 0.4	21.2 ± 2.3	68.3 ± 0.4	24.4 ± 0.1	7.3	2.80
A_1: ad libitum		5 ♀	42.0 ± 4.1	85.7 ± 4.5	31.0 ± 1.1	9.4 ± 0.6	25.0 ± 2.1	68.0 ± 0.6	24.6 ± 0.3	7.4	2.76

[1] Plus or minus values are the standard errors of the means.
[2] Per cent of total gain.
[3] Per cent of fat-free gain.

The lesser gain of fat (3.3 gm.) by the low-thiamine male rats was counterbalanced by a greater gain of water (2.4 gm.) and protein (0.9 gm.) in comparison with their high-thiamine pair-mates. Thus, the total gains of the high- and low-thiamine males were essentially the same.

The total gain of the high-thiamine females was 11.1 gm. greater than that of their low-thiamine pair-mates. This difference was evident in all five pairs and was highly significant statistically. Water (6.7 gm.) accounted for 60%, and protein (2.3 gm.) for 20% of the greater gain of the high-thiamine females. The ratio of water to protein (2.91) in the greater gain was normal.

The percentage of water and protein in the fat-free gains and the relationship of water and protein to each other were remarkably consistent for the high- and low-thiamine rats of both sexes. The slightly higher percentage of water in the fat-free tissue gained by the high-thiamine females in comparison with that of the low-thiamine females was highly significant statistically (odds 400 to 1) but its physiological significance, if any exists, is obscure.

Low riboflavin. The low-riboflavin rats of both sexes gained substantially less fat than did their high-riboflavin pair-mates. This difference accounted for 60% of the greater gain of the high-riboflavin males and all of the greater gain of the high-riboflavin females.

The high-riboflavin male rats gained slightly more water and protein than their low-riboflavin pair-mates, but no difference in these constituents was evident in the females.

Low pyridoxine. Most of the significant difference in total gain attributable to the effects of pyridoxine (Voris et al., '42) occurred in the male rats. However, the female rats conformed to the general pattern of differential composition resulting from the pyridoxine deficiency.

Pyridoxine, unlike thiamine and riboflavin, had no significant effect on the fat gained by either sex, but the pyridoxine-deficient rats gained less water and protein than did their high-pyridoxine pair-mates.

The relationships of the gain in water and protein to the fat-free gain, and to each other, were not significantly different in the high- and low-pyridoxine rats of either sex. In this respect, the composition of the greater gains of the high-pyridoxine rats was characteristic of normal growth.

In the male rats of this group the values representing the relationships of water and protein to the fat-free gain, and to each other, were consistent with the corresponding values obtained for the rats in the thiamine, riboflavin and pantothenate trials; but in the females of the pyridoxine group the percentage of water in the fat-free gain was slightly low, and the percentage of protein slightly high, in comparison with the relatively constant values for these relationships observed in the other groups. This deviation was common to both the high- and low-pyridoxine females and, hence, was not related to a pyridoxine deficiency. Since the total gain of the pyridoxine females was considerably less than that in any of the other groups, the results indicated a preferential retention of protein relative to water when the normal tendency to grow was limited.

As a result of this observation, the ratios of water to protein gained in individual rats, regardless of treatment, were arranged according to the protein gained (3 gm. limits) with the following results: when the range of protein gained was 13–20 gm., the water gained per gram of protein gained averaged 2.55; when the range of protein gained was 21–33 gm., the ratio was remarkably constant at 2.78; when the range of protein gained was 34–40 gm., the ratio was constant at 2.85. Of the ninety-five individuals, seven were included in the first range, sixty-five in the second range and twenty-three in the highest range. These results support the conclusion suggested above that when the growth impulse is restrained by limited food consumption the tendency to retain protein is greater than the tendency to retain water, and the characteristic relationship between these constituents is disturbed.

Low pantothenate. The average differences in total gains between the high- and low-pantothenate rats were 19.3 gm. in

the males and 16.0 gm. in the females. In this respect, the sexes reacted more nearly alike to pantothenate deficiencies than they did to deficiencies in thiamine, riboflavin or pyridoxine.

The absolute gains of fat, water and protein in the low-pantothenate rats were all significantly less than those of the high-pantothenate pair-mates. Fat accounted for 33%, water for 52% and protein for 12% of the greater gain of the high-pantothenate males, while in the female rats fat represented 57%, water 31% and protein 14% of the greater gain. Thus the composition of the gains promoted by pantothenate appeared to be characteristic for normal growth.

None of the four vitamins tested affected the gains of the non-protein, non-fatty dry tissue (residual gain) significantly. Thus, no effect of the B vitamins on skeletal growth was evident in the present investigation.

DISCUSSION

The primary effect of thiamine appeared in the fat content of the body. In male rats this was its only effect. The specific growth effect of thiamine in female rats may be associated with the characteristically high quota of body fat in this sex as compared with that of males.

The iodine number of body fats has been found to decrease significantly, from 77.4 to 72.1, as the level of thiamine was increased from 15 μg. to 60 μg. per day per rat (Voris and Thacker, unpublished results). This decline in iodine number of the body fats with increasing thiamine intake showed a directional similarity to the iodine number of the fats synthesized from carbohydrate under the influence of thiamine as reported by Longenecker, Gavin and McHenry ('40). However, the fact that the iodine number of the body fats approached that of the dietary fat, as the level of thiamine intake increased, suggested also the possibility that the extra fat gained by the rats on the higher level of thiamine was derived from dietary fat spared by the preferential utilization of carbohydrate.

Riboflavin, like thiamine, affected primarily the body fat content but the sexual differences were directly opposite to those observed in the thiamine test. A specific, but seemingly secondary, growth effect of riboflavin was evident in the male rats, whereas fat alone was affected in the females.

The promotion of fat deposition by riboflavin was observed by Sure and Dichek ('41), but the sexes were not differentiated, and it is not evident from their report whether the relatively smaller gain of protein accompanying the fat gain was characteristic for both males and females.

Riboflavin appears to play no part in the synthesis of fat from carbohydrate and protein (McHenry and Gavin, '38; '41), but its relationship to lipid metabolism has been emphasized by Mannering, Lipton and Elvehjem ('41), and by Shaw and Phillips ('41). From the present results it seems most likely that the extra fat gained by the high-riboflavin rats, compared with their riboflavin-deficient pair-mates, resulted from the sparing of dietary fat through more efficient utilization of dietary energy.

It is difficult to reconcile the present results with those of Kleiber and Jukes ('42) who found that the utilization of protein was decreased but that of food energy was not affected in riboflavin-deficient chicks.

Pyridoxine, like riboflavin, appeared to affect male rats more prominently than it did female rats with respect to the growth differences observed, though the appetite depression with a deficiency of either of these vitamins was more prominent in the females (Voris et al., '42).

In both sexes the primary effect of pyridoxine was associated with protein metabolism, a relationship which has been observed by McHenry and Gavin ('41) and by Foy and Cerecedo ('41).

Pantothenate seemed to be the only one of the four vitamins studied which had a general effect on the growth of rats uncomplicated by sexual differences or by specific effects on the composition of the body increase. In this respect the results conform to the suggestion of Unna and Richards ('42) that

pantothenic acid may be associated with the metabolic processes involved in the formation of new tissue. This speculation was based on the observation that the greatest need for pantothenic acid coincided with the period of maximal growth.

SUMMARY

The composition of the body gains of male and female rats receiving diets moderately deficient in thiamine, riboflavin, pyridoxine or calcium pantothenate was compared with the composition of body gains of rats pair-fed a similar diet adequately fortified with the respective vitamins. The gains were accounted for in terms of water, protein, fat, and residual substance.

In female rats thiamine promoted gains of fat, water and protein in relationships which were characteristic of normal growth. In male rats thiamine affected only the body fat content and had little or no effect on the fat-free tissue gained.

The primary effect of riboflavin was to increase the body fat. A specific, but seemingly secondary, growth effect of riboflavin was evident in male rats, whereas fat alone was affected in females. In this respect, the reactions of the sexes to thiamine and riboflavin deficiencies were directly opposite. The over-all effect of riboflavin was more prominent in male than in female rats.

Pyridoxine, like riboflavin, appeared to affect the male rats more prominently than it did the females. In both sexes the primary effect of pyridoxine was associated with the gains of water and protein, and there was no significant effect on the body fat content.

Pantothenate promoted the gains of fat, water and protein in proportions which were characteristic of normal growth for both sexes. Pantothenate was the only one of the four vitamins studied which had a general effect on growth uncomplicated by sexual differences and by specific effects on the composition of the body increase.

None of the four vitamins affected the gains of non-protein, non-fatty dry substance.

LITERATURE CITED

ADDIS, T., L. J. POO, W. LOO AND D. W. YUEN 1936 Gravimetric methods for the determination of total body protein and organ protein. J. Biol. Chem., vol. 113, p. 497.

FOY, J. R., AND L. R. CERECEDO 1941 The effect of protein on vitamin B_6 deficiency. Abstr., Div. Biol. Chem., Am. Chem. Soc., September meeting, p. 24.

KLEIBER, M., AND T. H. JUKES 1942 Metabolism and food utilization of riboflavin-deficient chicks. Proc. Soc. Exp. Biol. Med., vol. 49, p. 34.

LONGENECKER, H. E., G. GAVIN AND E. W. MCHENRY 1940 Fatty acids synthesized by the action of thiamine. J. Biol. Chem., vol. 134, p. 693.

LOVE, H. H. 1924 A modification of Student's table for use in interpreting experimental results. J. Am. Soc. Agron., vol. 16, p. 68.

MANNERING, G. J., M. A. LIPTON AND C. A. ELVEHJEM 1941 Relation of dietary fat to riboflavin requirement of growing rats. Proc. Soc. Exp. Biol. Med., vol. 46, p. 100.

MCHENRY, E. W., AND G. GAVIN 1938 The B vitamins and fat metabolism. I. Effects of thiamine, riboflavin, and rice polish concentrate upon body fat. J. Biol. Chem., vol. 125, p. 653.

———— 1941 The B vitamins and fat metabolism. IV. The synthesis of fat from protein. J. Biol. Chem., vol. 138, p. 471.

SHAW, J. H., AND P. H. PHILLIPS 1941 The pathology of riboflavin deficiency in the rat. J. Nutrition, vol. 22, p. 345.

SURE, B., AND M. DICHEK 1941 Riboflavin as a factor in economy of food utilization. J. Nutrition, vol. 41, p. 453.

UNNA, K., AND G. V. RICHARDS 1942 Relationship between pantothenic acid requirement and age in the rat. J. Nutrition, vol. 23, p. 545.

VORIS, L., A. BLACK, R. W. SWIFT AND C. E. FRENCH 1942 Thiamine, riboflavin, pyridoxine and pantothenate deficiencies as affecting the appetite and growth of the albino rat. J. Nutrition, vol. 23, p. 555.

THE INTERRELATION OF CALCIUM AND FAT UTILIZATION [1]

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Following an investigation reported by the author in a recent number of this Journal (French, '42) experiments were conducted for the purpose of supplementing the evidence presented in the earlier paper — specifically, by making observations on the effects of diets containing less than 4.7%, or more than 27.7%, of fat on the utilization of the calcium of equicaloric diets.

The earlier work gives results obtained with diets containing 4.7%, 14.0%, 27.7%, and 45.5% of fat, while the results here presented represent diets containing 0.28%, 1.9%, 34.0% and 39.3% of fat.

The general procedure in the present experiment was the same as that used in the earlier study, the results being derived from the analysis of the excreta from four groups of male rats of nine individuals each, each set of four being from one litter, and each group of nine being made up of one rat from each group of four litter-mates.

The diets, compounded as indicated in table 1, with fat contents as stated above, were so devised that when fed in equicaloric quantities, as in this experiment, they also supplied nitrogen, calcium and phosphorus at approximately constant levels of intake; the average daily gain in live weight of the four groups of rats on the diets of different fat content varied only between 1.85 gm. and 1.98 gm.

[1] Authorized for publication on June 13, 1942, as paper no. 1112 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

The numerical values for these dietary constituents are given in table 2 (experiment II); in the same table, for convenient comparison, are given data pertaining to the earlier experiment (experiment I).

With reference to the present (the second) experiment, the rats were all started on the special diets at 25 days of age; collection of excreta was begun 4 days later. The average initial weights of groups 5, 6, 7 and 8, at 29 days of age, were 58, 59, 58 and 56 gm., respectively.

TABLE 1

Composition of experimental diets.

COMPONENTS	DIET NO. 5	DIET NO. 6	DIET NO. 7	DIET NO. 8
	%	%	%	%
Dextrin [1]	76.82	74.57	31.44	24.29
Casein [2] (vitamin free)	17.65	18.15	26.41	27.79
Oleo oil [3] (+ vitamins)	....	1.89	33.97	39.29
Linoleic acid (+ vitamins)	0.28	....	....	....
Celluflour [4]	1.77	1.82	2.64	2.78
Salt mixture	2.66	2.72	3.96	4.17
$Ca_3(PO_4)_2$	0.82	0.85	1.30	1.36
K_2HPO_4	....	....	0.28	0.32
	100.00	100.00	100.00	100.00

[1] Amidex, from the Corn Products Refining Co.
[2] From the Casein Company of America.
[3] Oleo oil, Harrison Grade, of Wilson and Co.
[4] Chicago Dietetic Supply House.

The mineral salts fed were compounded as in Osborne and Mendel's XXX mixture; and for each 4350 Cal. of energy of each diet, vitamins were provided by the addition of the following compounds in the amounts specified: thiamine, riboflavin and pyridoxine 5 mg. each, calcium pantothenate 10 mg., viosterol 3500 I. U., β-carotene 2.78 mg. and choline 2.00 gm. In addition, to each 4350 Cal. of diet no. 5 (containing 0.28% fat) was added 25 mg. of α-tocopherol and 2.00 gm. of linoleic acid.

As in the earlier experiment the rats in the present study were killed at the end of the collection period, which in this

test was 27 days long, and the acidity of the alimentary tract was determined (table 3) in terms of pH values.

Also as in the earlier experiment, the acidity of the contents of the alimentary tract diminished in the order of the increase in the fat content of the diets.

TABLE 2

Fat, calcium and nitrogen contents of diets, average daily intake of food and of nutrients, and gain in weight of rats.

EXPERIMENT AND DIET NO.	FAT CONTENT OF DIET	Ca CONTENT OF DIET	N CONTENT OF DIET	DAILY FOOD	DAILY FAT	DAILY Ca	DAILY ENERGY	DAILY GAIN IN WEIGHT
	%	%	%	*gm.*	*gm.*	*mg.*	*Cal.*	*gm.*
Experiment I[1]								
1	4.72	3.03	2.79	6.31	0.305	19.1	25.4	1.61
2	14.04	3.31	3.14	5.59	0.755	18.5	25.3	1.67
3	27.74	3.96	3.61	4.80	1.255	19.0	25.2	1.67
4	45.46	5.38	4.33	3.29	1.497	17.7	22.3	0.74
Experiment II								
5	0.28	3.19	2.46	7.45	0.027	23.8	30.0	1.95
6	1.89	3.29	2.74	7.26	0.154	23.9	30.0	1.85
7	33.97	4.80	3.80	4.98	1.690	23.9	29.7	1.97
8	39.29	5.04	4.14	4.74	1.816	23.9	29.8	1.98

[1] From J. Nutrition, vol. 23, p. 375 (1942).

TABLE 3

Average pH values of intestinal contents at end of experiment II.

DIET NO.	NUMBER OF RATS	DUODENUM	JEJUNUM	ILEUM	CECUM
		pH	*pH*	*pH*	*pH*
5	9	5.49	5.75	7.24	6.47
6	9	5.69	5.75	7.38	6.59
7	9	5.94	6.32	7.31	6.93
8	9	6.04	6.45	7.40	6.94

As to the relation of dietary fat to calcium utilization (table 4), the two experiments will be considered separately since, as shown in table 2, the daily intake of energy and of calcium, and the average daily gains in body weight, differed materially in the two studies.

Further, it is necessary to reconsider the performance in the first experiment of the group of rats on diet no. 4. The animals in this group were fed ad libitum because they were unable to consume their diet containing 45.40% of fat, in quantities equivalent to those eaten by the other groups; they gained less than half as much in weight as did any other group of rats in either experiment.

In view of the low plane of nutrition of this group of rats, the low rate of efficiency with which they utilized calcium must be ascribed primarily to the combination of conditions which limited the growth rather than to the fat content of the diet. This group of rats, therefore, was eliminated from further consideration.

TABLE 4

Average daily calcium balances.

	DIET NO.	FAT CONTENT OF DIET	CALCIUM				
			Intake	In urine	In feces	Balance	Retention
		%	*mg.*	*mg.*	*mg.*	*mg.*	%
Experiment I[1]	1	4.72	19.1	0.40	3.43	15.3	80
	2	14.04	18.5	0.38	3.75	14.4	78
	3	27.74	19.0	0.45	4.48	14.1	74
	4	45.46	17.7	0.43	9.00	8.3	47
Experiment II	5	0.28	23.8	0.52	4.46	18.8	79
	6	1.89	23.9	0.57	5.16	18.2	76
	7	33.97	23.9	0.42	5.38	18.1	76
	8	39.29	23.9	0.50	5.49	17.9	75

[1] From J. Nutrition, vol. 23, p. 375 (1942).

From the two experiments the statistical evidence in favor of the idea that an increasing fat content of equicaloric diets militates against the efficiency of utilization of calcium is expressed by odds of 5:1 between diets 1 and 2; 76:1 between diets 1 and 3; 108:1 between diets 5 and 6; 20:1 between diets 5 and 7; and 28:1 between diets 5 and 8.

This finding appears to be in opposition to the observations of Jones ('42) who reported beneficial effects of lard and oleic acid in relation to calcium utilization. This difference in

results may have been due to the difference in the kind of fat used, or to the fact that in Jones's experiment the fat was added to a vitamin D-free diet, while in the experiment here discussed vitamin D was supplied in the basal diet.

CONCLUSIONS

The evidence presented indicates a slight interference of oleo oil with calcium utilization by the albino rat.

The acidity of the contents of the duodenum, jejunum, ileum and cecum was, in general, diminished by increase in the fat content of the diet, this decrease in intestinal acidity being associated with decrease in retention of calcium.

LITERATURE CITED

FRENCH, CYRUS E. 1942 The interrelation of calcium and fat utilization in the growing albino rat. J. Nutrition, vol. 23, p. 375.

JONES, J. H. 1942 The relation of the pH of intestinal contents to calcium and phosphorus utilization. J. Biol. Chem., vol. 142, p. 557.

ERRATUM

FRENCH, CYRUS E. 1942 The interrelation of calcium and fat utilization in the growing albino rat. J. Nutrition, v. 23, no. 4, April 10.

Page 377, starting line 3, change to read: "Salts were provided in the Osborne and Mendel XXX mixture."

Omit the following: "as modified by Wesson ('32), and as further modified by eliminating its contents of calcium and phosphorus."

A SHORT METHOD OF CALCULATING THE NUTRITIVE VALUE OF DIETS

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The outstanding nutritional problems of today are the subclinical rather than the obvious or extreme deficiency states. The various symptoms of these mild deficiencies, such as vague ill-health and malaise characterized by lassitude, weakness, lack of stamina, and a general lowering of morale, while obviously undesirable in the civilian, take on added significance when they appear in the soldier. In terms of effective manpower, they can be decisive factors in modern war. Workers in military preventive medicine, aware of the importance of optimal nutrition, have become vitally interested in the assurance of an adequate diet for the soldier, and recognize that proper nutrition is dependent primarily upon two factors, (a) the proper planning of meals, and (b) the conservation of the inherent nutritive value of all foodstuffs prepared for those meals. It is no longer sufficient merely to plan a series of menus which are nutritionally adequate and well-balanced; it is of equal, if not greater, importance to avoid all practices which adversely affect nutritive value, and therefore decrease the physiologic value of foods provided for a carefully planned menu. Prolonged heating of food (particularly meat) at high temperatures, oversoaking of vegetables and discarding of their cooking water, preparation of meals too far in advance of the scheduled meal time, all these, and many other improper practices serve to prevent the attainment of the value of food

as planned. Food and Nutrition Officers of the Sanitary Corps are therefore placing emphasis on the avoidance of all such practices, in order that the planned adequacy of the soldiers' diet may be attained in fact when the food is actually consumed.

The establishment of quantitative nutritional daily requirements by the Food and Nutrition Board of the National Research Council has presented quantitative problems to those who are concerned with health as it may be affected by nutrition. Where large masses of individuals are concerned, such as the Army and Navy, a nutritional accounting of food is not only important from the standpoint of health and effectiveness, but it is also amply justified by the numbers involved. Yet the process of adding together the nutritive values of all the servings of daily food over any appreciable length of time would be such a tedious problem that, for practical purposes, it would be almost impossible. The need for a rapid and fairly accurate method of determining the approximate nutritive value of diets has therefore been great, and it is the purpose of this paper to present such a method. The principles of this method have been used for some time in the Bureau of Home Economics by Dr. Hazel K. Stiebeling, although the method itself has not been previously described or published in detail.

The present method is intended for use in connection with a procedure used successfully by Col. Paul E. Howe, Sanitary Corps, for nutritional accounting of mass feeding. The procedure is based on the grouping of foods into classes on the basis of (1) similar nutritive content, (2) special function in the diet, and (3) unique contribution to the value of the diet. On these bases all foods, either canned or fresh, fall into seventeen classes as follows: meats, fish and poultry; eggs; milk and milk products; butter; fats other than butter; grain products; legumes; sugars and syrups; leafy green or yellow vegetables; tomatoes; citrus fruits; potatoes; vegetables other than leafy green or yellow; fruits other than citrus; dried fruits; beverages; and miscellaneous items. The quantities of food purchased or consumed, when so classified and reduced

to a unit basis of pounds per man per day, can be used as a first approximation for judging the adequacy of the dietary, and can indicate whether or not further analysis is necessary should there be a suspicion of actual inadequacy of one or more nutritive constituents. This procedure constitutes, in effect, a means of nutritional accounting, which is of equal or greater importance than the customary cost accounting. Through such a method one recognizes that adequate dietaries may be obtained from a variety of foods, and varying quantities of any of the classes of foods. In effect, nutritional adequacy is attainable by a variety of means, and what may appear to be a deficiency in the quantity of one class may be offset by an increased quantity of another class. For example, citrus fruits and sufficient leafy green or yellow vegetables are approximately interchangeable with regard to vitamin C.

Once having placed the foods of a diet into their appropriate classes (Howe, Pritchett and Berryman, '42), it is a simple mathematical procedure to arrive at the pounds per man per day intake of each class of food over any period of time (preferably 10 days at least). Such a calculation can be made on either the quantities of food planned for a menu, or on the quantities of food actually used in the kitchen to prepare the meals.

This classification contains certain exceptions from previous methods of grouping, particularly with regard to the classification of sweet potatoes, tomatoes, citrus fruits, jams, jellies, and gelatin dessert powders. The last three are classed with "sugars and syrups" because of their high sugar content. Tomatoes and citrus fruits are divided into separate classes because, while it is true that, together, they are good sources of ascorbic acid, yet, on the other hand, tomatoes are usually appreciably higher with regard to pro-vitamin A content. The first of these exceptions, namely, that sweet potatoes are now grouped with the leafy green or yellow vegetables rather than with the white or Irish potatoes, deserves special mention, however. From the standpoint of alternative use on the menu,

it might appear more logical to group the sweet potatoes with white potatoes. On the other hand, for purposes of calculation of the nutritive value of diets, the contrast in pro-vitamin A content between the yellow sweet and the white potatoes is so great that serious error in evaluation of vitamin A activity would result by grouping them together. Such an error, due to deviations in the proportion of sweet potatoes, was potentially so great that to prevent it would have required a separate record of the intake of sweet potatoes, and an independent estimate of its pro-vitamin A which, in effect, would be to add to the number of food classes. With the present system of classifying them with leafy green or yellow vegetables, because of the general similarity in nutritive content, no separate records or calculations on sweet potatoes are needed.

The next step is to apply weighted nutritive values (table 1) for the various classes of food to the computed intake of each class (pounds per day per man). For example, if it is determined that the computed average daily intake of the class "meats, fish, and poultry" is 0.75 pound per day per man, then the group value per pound for "meats, fish, and poultry," in terms of protein, fat, carbohydrate, minerals and vitamins, can be multiplied by 0.75, and the average daily contribution of this class determined. Appropriate group values for each food class are similarly applied and the sums of the products for each nutritive component represent the approximate nutritive value of the average daily food for the period concerned (table 2).

The validity of the method obviously depends upon obtaining group nutritive values for protein, fat, carbohydrate, minerals, and vitamins, which properly represent the food habits involved. The frequency of use of each food determines such a food pattern; therefore, the food habits of the Army have been studied by considering the menus of representative camps during 6 months, May through October, 1941 (Field Ration A).[1] The planned intake of each food in camps all

[1] The ration now generally in use by the U. S. Army within the continental United States; characterized by a wide choice of foods, both fresh and canned, and corresponding roughly to a fairly liberal civilian diet.

TABLE 1

Short method of computing nutritive value of ration.

Nutrients per pound of food groups "as purchased" (based on food prescribed for U. S. Army May–October, 1941)

GROUP	CALORIES	PROTEIN	FAT	CARBO-HYDRATE	Ca	P	Fe	VITAMIN A	THIAMINE	RIBO-FLAVIN	NICO-TINIC ACID	ASCORBIC ACID
		gm.	*gm.*	*gm.*	*mg.*	*mg.*	*mg.*	*I.U.*	*mg.*	*mg.*	*mg.*	*mg.*
Meats, fish and poultry	1120.	65.	95.	2.	41.	719.	9.8	120.[1]	1.87 [2]	.77 [1]	22.9	3.
Eggs, fresh	635.	52.	46.	3.	218.	848.	10.9	4,040.	.83	1.45	.2	0.
Milk products (equivs.)	360.	16.	21.	25.	556.	427.	.9	1,020.	.11	.74	.4	4.
Butter	3320.	3.	367.	2.	73.	73.	.9	14,970.	.01	.04	.5	0.
Fats, other	3900.	1.	432.	3.	2.	9.	.1	120.	.01	.01	.2	0.
Grain products	1370.	40.	14.	270.	160.	482.	5.5	20.	1.08 [2]	.29	4.2	0.
Legumes, dry	1630.	97.	15.	276.	538.	1968.	40.7	10.	2.40	1.33	12.5	0.
Sugars and syrups	1680.	1.	0.	420.	37.	16.	2.2	0.	.00	.00	.0	0.
Vegetables, leafy green or yellow	150.	7.	1.	30.	145.	152.	3.5	14,750.	.29	.24	2.7	91.
Tomatoes	130.	5.	1.	26.	61.	160.	2.6	3,860.	.36	.20	2.2	78.
Citrus fruits	140.	2.	1.	32.	91.	66.	1.2	90.	.23	.07	1.5	118.
Potatoes, white	330.	8.	0.	73.	30.	186.	2.8	150.	.34	.19	4.5	38.
Vegetables, other	180.	6.	1.	37.	102.	173.	1.9	180.	.12	.22	.9	49.
Fruits, other than citrus, fresh and canned	290.	2.	1.	69.	34.	51.	1.3	1,940.	.09	.17	1.4	24.
Fruits, dried	1280.	12.	2.	302.	245.	452.	18.0	8,320.	.47	1.63	6.6	0.
Total												

[1] When liver is used add 125,000 I.U. vitamin A and 15.56 mg. of riboflavin per pound of liver.

[2] Use this figure only when the combined weight of the lean pork cuts (viz., fresh and cured ham, pork loin and chops, Boston butt, and shoulder) is 15% or more of the weight of the meat group. When the combined weight is less than 15%, compute the thiamine thus: [wt. (in lbs.) of lean pork cuts × 5.9] plus [wt. (in lbs.) of all other meat × 1.0] is quantity of thiamine in milligram in the entire group.

[3] When enriched flour and bread are not used, count thiamine in the entire group as only 0.59 mg. per pound (based on "straight" flour); no changes need be made in other nutrients (unless riboflavin is added to enriched flour).

TABLE 2

Nutritive value per man per day, station 1 (July, 1941).

Computed by the short method.

GROUP	WT. IN LBS.	CALORIES	PROTEIN	FAT	CARBO-HYDRATE	Ca	P	Fe	VITAMIN A	THIAMINE	RIBO-FLAVIN	NICOTINIC ACID	ASCORBIC ACID
			gm.	*gm.*	*gm.*	*mg.*	*mg.*	*mg.*	*I.U.*	*mg.*	*mg.*	*mg.*	*mg.*
1. Meats, fish and poultry [1]	.911	1020.	59.	87.	2.	37.	655.	8.93	109.	1.352 [2]	.701	20.86	3.
2. Eggs, fresh	.190	121.	10.	9.	1.	41.	161.	2.07	768.	.158	.276	.04	0.
3. Milk products (equivs.)	.976	351.	16.	20.	24.	543.	417.	.88	996.	.107	.722	.39	4.
4. Butter	.081	269.	0.	30.	0.	6.	6.	.07	1,213.	.001	.003	.04	0.
5. Fats, other	.067	261.	0.	29.	0.	0.	1.	.01	8.	.001	.001	.01	0.
6. Grain products	.708	970.	28.	10.	191.	113.	341.	3.89	14.	.418 [3 4]	.205	2.97	0.
7. Legumes, dry	.073	119.	7.	1.	20.	39.	144.	2.97	1.	.175	.097	.91	0.
8. Sugars and syrups	.318	534.	0.	0.	134.	12.	5.	.70	0.	.000	.000	.00	0.
9. Vegetables, leafy green and yellow	.495	74.	3.	0.	15.	72.	75.	1.73	7,301.	.144	.119	1.34	45.
10. Tomatoes	.121	16.	1.	0.	3.	7.	19.	.31	467.	.044	.024	.27	9.
11. Citrus fruits	.281	39.	1.	0.	9.	26.	19.	.34	25.	.065	.020	.42	33.
12. Potatoes, white	.513	169.	4.	0.	37.	15.	95.	1.44	77.	.174	.097	2.31	19.
13. Vegetables, other	.358	64.	2.	0.	13.	62.	62.	.68	64.	.043	.079	.32	18.
14. Fruits, other than citrus fresh and canned	.389	113.	1.	0.	27.	13.	20.	.51	755.	.035	.066	.54	9.
15. Fruits, dried	.020	26.	0.	0.	6.	5.	9.	.36	166.	.009	.033	.13	0.
Subtotal used as short method (15 food classes)	5.501	4146.	132.	186.	482.	966.	2029.	24.89	11,964.	2.726	2.443	30.55	140.
16. Beverages (dry)	.124	14.	0.	1.	1.	5.	29.	.11	0.	.002	.000	.00	0.
17. Misc. incl. yeast	.112	4.	0.	0.	1.	0.	5.	.06	2.	.008	.016	.09	0.
Total (17 food classes)	5.737	4164.	132.	187.	484.	971.	2063.	25.1	11,966.	2.74	2.46	30.6	140.
Error in per cent from excl. last 2 groups)	4.1	4.3	0.	.5	.4	.5	1.6	.07	.02	.004	.7	.3	0.

[1] Including .090 pound lean pork; .821 pound of other meat; no liver.

[2] Derived as follows: .090 lb. at 5.9 = .531
.821 lb. at 1.0 = .821
1.352

The total by the long method for the meat group is 1.362 mg. If all meat had been calculated by 1.87 mg. per pound, the total for meat would have been 1.704 (25% higher). Loss in cooking (see text p. 31) is counted as .676 mg. (50% of 1.352).

[3] Flour and bread were not enriched.

[4] As the long method gave .465 mg. for this class, a difference of —.047 mg. was introduced by the short method. In this ration, the error in the grain group is approximately balanced by the combination of errors in two other groups.

over the country has been determined and weighted to take into account the number of troops subsisting according to each menu. Thus, it was found that the planned Army consumption of carcass beef during those months stood at 18.86% of the entire intake of the group "meats, fish, and poultry," and similarly, percentages for all the other meat, fish, or poultry items in this group were calculated. Likewise, of the planned consumption of "leafy green or yellow vegetables," cabbage comprised 22.11%, carrots 11.04%, and so on, for each other item within the group. The percentage contribution of each food to the weight of the planned intake of its food group thus determined the food pattern; this, in turn, became the basis for the group nutritive values. These particular values may, for convenience, be termed "summer" values; a similar pattern is being developed for the "winter" months, November, 1941, through April, 1942.

As there have been rapid developments in the knowledge of methods of analysis, and much new information obtained on the composition of foods, especially with regard to vitamin content, it was essential as a step in developing the figures for the nutritive values of each group to select the most authentic of the recent findings for each food item. The Bureau of Home Economics therefore developed average figures for the proximate, mineral, and vitamin content of about 150 of the food items that were used in largest quantities in Army Field Ration A during the 6 months stated above. For these items, available published or unpublished values were assembled and evaluated, relying chiefly on Bureau of Home Economics summaries of acceptable source material. For the remaining items, about seventy in number, consisting chiefly of food mixtures and items consumed by the Army in comparatively small quantities, values from standard published tables were used. Data on nicotinic acid content were taken almost entirely from a compilation by McVicar and Berryman ('42). Conservative values were imputed for a number of food items not yet reported.

In selecting the figures for each of the items, care was taken, in so far as available data permitted, to choose the grade, kind, and form of each of the foods appropriate to Army habit and custom. Army specifications for purchasing the various products were studied to determine which of several alternative figures would be the most appropriate. For example, medium fat beef, expressed in terms of the weight "as purchased" was considered to be appropriate for the grades of beef purchased as carcasses, as boneless carcasses, or as wholesale cuts. In the case of poultry, average roasters were chosen as having a composition suitable to represent the combination of fryers, roasters, and fowls purchased by the Army; the weight "as purchased" was considered to be the weight of the dressed, but not drawn, poultry. Likewise, figures for "straight" flour, and for bread made from this kind of flour according to the "Army Baker" recipe, were obtained in preference to figures for ordinary white flour and white bread.

There was less option in selecting the right figure for the vitamins, since vitamin data are less numerous. However, selection was often possible, even here, when the choice was between two kinds, either of which may be used, or between two or more observations on the general type; in such a case either an average, or else the more conservative value, was selected.

Weighted average figures per pound for the various food groups were then computed, as previously mentioned. The figures in table 1 correspond with the weight of the group "as purchased" and with the condition of the foods "as purchased," viz., as received at the mess kitchen. For example, the vitamin values are designed to correspond with the vitamin content of the food as received whether raw, canned, or cured, before the foods are cooked and prepared for the table. Allowances for average loss as "refuse," i.e., inedible material, or material not generally served or eaten, are contained in the figures presented. Apart from "refuse," neither losses in preparation nor table waste of edible material is provided

for, nor has any allowance been made for the destruction of vitamins during long storage, cooking, or serving.

In this latter regard, however, and as a final step in the evaluation, it is possible to make separate deductions for probable conservative losses in cooking and preparation. In so far as our present knowledge permits, these have been discussed by Howe and Berryman ('42) and the following tentative deductions are suggested: about 50% of the thiamine of "meats, fish, and poultry" (Army methods of cooking); 25% of the thiamine of cooked vegetables, with an additional 20% lost if the cooking liquors are discarded; 50% of the vitamin C of cooked vegetables (except tomatoes); and 25% of the vitamin C of fresh vegetables or fruits cut up and consumed raw in salads or desserts. The deductions, even though they may be considered conservative, have a serious effect upon the value of the food as planned. For instance, when these losses are applied to the evaluation of the 6 months' pattern (table 3) the net effect is to lower the average probable thiamine intake from about 3 mg. as planned to about 2 mg. as probably consumed, and similarly to decrease the ascorbic acid from about 132 mg. to approximately 97 mg. Moreover, it must be emphasized that drastic methods of preparation and cooking would lower these probable consumption levels still further.

In order to test the validity of this short method, the monthly menus from three stations were evaluated by it. The results obtained were then compared with those calculated by the much longer, i.e., the conventional method of applying average individual values to each average serving of each food used, with the grand total computed therefrom. These menus were chosen to represent maximum deviation in the proportions of liver and of sweet potatoes, since these two foods appeared at first to be the ones that would give rise to maximum deviations in the nutritive value of the group and, therefore, would require the most detailed consideration. It was hoped, too, that rations selected on this basis would deviate from the 6-month pattern for the United States Army in

TABLE 3

Comparison of methods (quantities per man per day).

Results by short method at 3 stations compared with more detailed estimates by long method and with U. S. Army 6 months' period and National Research Council recommended allowances.

	CALORIES	PROTEIN	FAT	CARBO-HYDRATE	Ca	P	Fe	VITAMIN A	THIAMINE	RIBO-FLAVIN	NICOTINIC ACID	ASCORBIC ACID
		gm.	*gm.*	*gm.*	*mg.*	*mg.*	*mg.*	*I.U.*	*mg.*	*mg.*	*mg.*	*mg.*
Station 1 — July, 1941												
Short method	4150	132.	187.	482.	966.	2030.	24.9	12,000	2.73	2.44	30.6	140.
Long method	4260	134.	192.	499.	978.	2050.	24.6	13,500	2.76	2.48	28.5	134.
Error	—110	—2.	—5.	—17.	—12.	—20.	+.3	—1,500	—.03	—.04	+2.1	+6.
Error in per cent [1]				—3.4%				—11.1%			+7.4%	+4.5%
Station 2 — June, 1941												
Short method	3880	127.	182.	428.	925.	1940.	23.2	11,400	3.04	2.39	31.7	132.
Long method	3840	127.	180.	424.	930.	1920.	22.3	11,300	3.23	2.41	30.8	132.
Error	+40	0.	+2.	+4.	—5.	+20.	+.9	+100	—.19	—.02	+.9	0.
Error in per cent [1]							+4.0%		—5.9%			
Station 3 — May, 1941												
Short method	4700	137.	199.	584.	912.	2060.	26.2	15,500	3.23	2.89	32.7	120.
Long method	4770	138.	211.	586.	936.	2020.	26.5	14,600	3.65	2.75	32.5	142.
Error	—70.	—1.	—12.	—2.	—24.	+40.	—.3	+900	—.42	+.14	+.2	—22.
Error in per cent [1]			—5.7%					+6.2%	—11.5%	+4.9%		—15.5%
U. S. Army avg., 6 mos. (May–Oct., 1941) [2]	4330	131.	195.	513.	1012.	2030.	24.5	12,900	2.98 [3]	2.67	29.6	132.
Recommended daily allowances [4]	4050	70.			800.		12.	5,000	2.15	3.12	21.5	75.

[1] Expressed in per cent of results by long method. Only errors greater than 3% are presented.

[2] Before subtraction of cooking losses. For discussion of estimate of losses see text page 31.

[3] Calculated on straight flour basis; enriched flour not used during this period.

[4] Adapted from the National Research Council standards on the basis of three-tenths of the time on moderately active duty and seven-tenths very active duty. These values apply to the value of the food actually ingested; whereas the other data apply to food as purchased, and before deductions are made for losses in preparation and cooking.

several other respects, thus offering severe tests of the accuracy of the method. The figures for one of these menus, station 1 in the southeast, obtained by the short method, are presented class by class in table 2, and the consolidated results for the average day are shown in table 3 together with a summary of data on the two other camps, the estimates by the long method being given for comparison. Table 2 also offers evidence that food groups no. 16 and no. 17, i.e., beverages and miscellaneous items, will rarely, if ever, contribute enough of any of the nutritionally important constituents to warrant their routine calculation. The authors therefore recommend omitting these classes from regular calculations, thereby saving time with practically no loss in accuracy. The number of important food classes is thus reduced from seventeen to fifteen for all practical purposes of calculating nutritive values.

After detailed comparison of the above diets it appeared that the use of the short method gives a substantially correct estimate of the diet as a whole. Three other rations were then chosen for further testing with respect to the leafy, green or yellow vegetable group. These, with nine additional rations, were also used for more detailed study of thiamine in meats. Here, no effort was made to select rations that were atypical. These were chosen rather on a geographical basis; the fifteen in all that were included in the study of thiamine in meats cover seven of the nine corps areas in the country.

DISCUSSION

Probable accuracy of the method

In the following discussion, the term "error," unless otherwise indicated, refers to that introduced by grouping the foods, i.e., the difference obtained by subtracting the long method evaluation from the short. This error is, of course, in addition to those inherent generally in any calculation based on "average" values.

The degree of error (greater than $\pm 3\%$) introduced by the use of the short method is illustrated in table 3. Negative signs obviously indicate an underestimate by the short method,

as purchased, and before deductions are made for losses in preparation and cooking.

and are accordingly in the right direction; positive signs are serious in that they indicate an overestimate, and might cause false confidence to be placed in the value of the diet.

General. For appraising calories, protein, fat, carbohydrates, minerals and vitamin A, the errors are not serious, at least with diets as liberal as Field Ration A. Errors within 10% are important only when the margin of safety, in the supplied quantity of the nutrient in question, is narrow. Since the average values for particular foods are themselves not too accurate, the risk of adding further errors in the order of 5%, through use of the short method, should not be regarded as invalidating the method.

Thiamine. Apart from meats, the thiamine estimates by the short method were very satisfactory, the difference noted in the grain group in station 1 being the widest of any in the three rations studied in full detail. Using the alternatives described below for estimating thiamine in meats brings this estimate within safe limits of error.

The estimation of thiamine in meats by any single value depends for its accuracy on the quantity of lean cuts of pork in the meat group more than on any other item or combination of items within the group. The widest discrepancies between the long method and the short, using the value 1.87 mg. per pound of "meat, fish, or poultry," were in the magnitude of 0.4 mg. per day, viz., about 18% of the day's allowance. The standard error based on all fifteen rations studied, was 0.22 mg. Since the meat group contributed most of the thiamine in the whole diet (often about half), the errors in this group would seldom be greatly modified by the combined errors in other groups. Of the fifteen rations that were studied, ten were overestimated by applying the single thiamine value (i.e., 1.87 mg. per pound) to the entire meat group, and three of these ten were overestimated by more than 0.3 mg. per day. For the remaining five, the single value was on the safe side, that is, the results were lower than those yielded by the long method.

Errors in this order of magnitude must be avoided, and the variation as described in footnote 2, table 1, was therefore developed. This variation, viz., the use of two values for subdivisions of the meat group, must be used under certain stated conditions. The reason that the variation is sometimes needed is that meat from the several species of animals is extremely variable in its thiamine content, and the proportions of the various kinds in use do not follow any general pattern very closely. This is important when it is considered that the more recent values for thiamine in the lean cuts of pork are in the order of 1.0 to 2.0 mg. per 100 gm., or 4.5 to 9.0 mg. per pound, while those for lean beef are about one-tenth this amount, and fish and poultry are still less. Fatter cuts of pork, and most of the sausages are lower in thiamine on the average than the lean pork, but appreciably higher than beef, veal, or lamb. Distribution within the meat group must accordingly be very regular to warrant placing reliance on a single weighted average value.

The variation mentioned above has been found to be essential whenever the consumption of lean pork cuts (viz., fresh and cured ham, pork loin and chops, Boston butts, and shoulder) is less than 15%. It consists of computing separately the combined weight of these six pork cuts at the rate of 5.9 mg. of thiamine per pound, and all the remainder of the meat group at 1.0 mg. per pound. When these pork cuts make up 15% or more of the meat group, however, the modification should be avoided. Then the single value as given in the table is preferable and will give either a good estimate, or an underestimate.

When the method is followed exactly, that is, using the above variation if lean pork is less than 15% of the total meat group, and the value 1.87 mg./lb. otherwise the results in general can be counted on as being conservative, with no overestimates greater than 0.2 mg. per day, judging by the rations on which the estimates were tested. The standard error in these fifteen was 0.16 mg. of thiamine per day. Only three of the fifteen rations studied were overestimated by the

recommended procedure, and these were to the extent of 0.05, 0.09 and 0.18 mg. per day.

Ascorbic acid. Ascorbic acid will seldom be overestimated by more than 10% before cooking, and this generally is not serious in appraising diets as liberal as the field ration, since this error is so much smaller than the potential errors in estimating the quantities lost in cooking. On the other hand, marked deviations from the 6-month pattern, particularly in the leafy green or yellow vegetable group, can introduce overestimates if particular items that are rich in ascorbic acid are not used, or are used in reduced amounts.

Riboflavin. Riboflavin estimation by the short method will usually come within 5% of that estimated by the long method. Even a 5% avoidable error may be important when the quantity supplied is low in relation to standard allowances. Table 3 shows that the 6 months' average furnished about 2.8 mg. before cooking, and no losses from cooking are computed. None of the rations studied in detail came up to the quantity needed on the basis of the National Research Council's recommendations. The conclusion that these diets afford no factor of safety follows from calculations by either the long or the short method. It is admitted that there may not be actual need for this amount of riboflavin, that is, the "yardstick" allowance may be somewhat high, but, until the standard is changed, 3.1 to 3.2 mg. should be the objective aimed at. In order to attain this level, it would therefore appear that added emphasis might well be placed on foods rich in riboflavin, such as liver, eggs, and brewers' yeast. Another alternative is to add riboflavin to enriched flour.

SUMMARY

For appraising Army Field Ration A, this short method of calculating the nutritive value of diets is adequate and a valuable timesaver. It should be useful for a rough approximation of the nutritive value of other liberal diets relatively unrestricted as to kinds and forms of foods. In its application elsewhere, however, it may require adaptation to fit the food

habits or pattern of the group under investigation. Properly adapted, this general method is a valuable means of obtaining a rough measure of the probable nutritional value of the diet concerned.

LITERATURE CITED

HOWE, P. E., AND G. H. BERRYMAN 1942 Outline for mess inspection. Army Medical Bulletin, no. 63, p. 151.

HOWE, P. E., C. S. PRITCHETT AND G. H. BERRYMAN 1942 Classification of foods, and factors for conversion of their packaging units to pounds. J. Am. Diet. Ass., vol. 18, p. 435.

MCVICAR, R. W., AND G. H. BERRYMAN 1942 The nicotinic acid content of foods. J. Nutrition, vol. 24, p. 235.

THE UTILIZATION FOR HEMOGLOBIN REGENERATION OF THE IRON IN SALTS USED IN THE ENRICHMENT OF FLOUR AND BREAD

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In the enrichment of flour according to Federal specifications, the addition of iron is a more difficult problem than the addition of thiamine and nicotinic acid (Tobey and Cathcart, '41). On the one hand, if the iron salt is not entirely insoluble, it causes adverse reactions, such as rancidity, in the flour; while, on the other hand, if it is not readily assimilable in the body, the purpose of its incorporation into flour may be largely defeated. According to Tobey and Cathcart, the iron salts commonly used in the enrichment of flour are iron phytate, sodium iron pyrophosphate and ferrum reductum. Since very little, if any, information has been reported on the biological utilization of the iron in these three combinations, it was the purpose of the experiments here reported to provide such information by feeding experiments designed to measure quantitatively the rates of hemoglobin regeneration induced by these iron salts in rats previously made anemic by milk feeding, as compared, in each case, with the rate induced by equivalent amounts of ferric chloride.

METHODS

The iron phytate used in the experiments is a commercial product prepared specifically for flour enrichment. According to the manufacturer,[1] the product is prepared from calcium

[1] Corn Products Refining Company, Argo, Illinois. Information kindly supplied by Mr. J. Paul Bishop, Director of the Research Department.

phytate by precipitating with an excess of ferric chloride. The constitution of the precipitate is not exactly known, though it appears to consist of a combination of ferric phytate with small amounts of calcium phytate and some water of hydration. The sample used in the experiments to be described contained on the dry basis (2 hours heating at 105°C.) 17.73% of iron, 19.09% of phosphorus, and 1.28% of calcium. It contained insignificant amounts of iron soluble in water or dilute acid.

The sodium iron pyrophosphate used is a double salt of sodium pyrophosphate and iron pyrophosphate containing 2 moles of the former to 1 of the latter.[2] Its formula is $Na_8Fe_4(P_2O_7)_5 \cdot 6H_2O$. The observed and theoretical composition indicated 16.08 and 16.12% of iron, 21.52 and 22.39% of phosphorus, and 8.23 and 7.79% water of hydration, respectively.

The ferrum reductum was a very pure reduced iron, U.S.P. grade, containing by actual analysis 100% of iron within the limits of error of the method.

The utilization of the iron of these three compounds for the regeneration of hemoglobin in the anemic rat was compared in turn with the utilization of the iron in ferric chloride prepared from standard iron wire.

At the age of 2 weeks, the experimental litters with the mother rats were placed in glass cages and fed mineralized milk (Kemmerer, Elvehjem, Hart and Fargo, '32) containing added copper, manganese and cobalt only, together with skim milk powder. When the young rats became sufficiently anemic they were paired for experimental feeding according to litter membership, sex, body weight and hemoglobin level. Eight to eleven pairs were used in each comparison. The experimental diet consisted of mineralized milk as described above, containing an average of 0.320 mg. of iron per liter, and skim milk solids, containing 0.000362% of iron. One gram of skim milk powder was fed daily to each rat, and in this portion

[2] Information kindly supplied by Mr. Howard Adler, Chief Chemist, Victor Chemical Works, Chicago, Illinois, manufacturer of the product.

was incorporated the iron salt under test, in an amount to provide, generally, 0.2 mg. of iron. Only after this mixture was consumed were the rats given their daily portions of mineralized milk. The intake of mineralized milk was equalized between pair mates in accordance with the appetite of the poorer eater. Such control of milk consumption in comparative tests of the availability of iron from different sources is essential, since it has been shown that a variable consumption of milk, even though the intake of test sources of iron is the same, induces a variable rate of hemoglobin regeneration (Mitchell and Hamilton, '37). For example, the sex difference noted in the rate of hemoglobin regeneration on equal intakes of iron (Rose and Hubbell, '38) disappears entirely when the intake of food is equalized also (Kinder, Mueller and Mitchell, '42).

Blood samples were taken weekly from the tails of the rats and analyzed for hemoglobin by the Sanford and Sheard ('30) method adapted to rat blood. At the termination of the feeding experiments, which lasted generally 3 or 4 weeks, the carcasses of some of the rats were analyzed for iron by the Stugart ('31) modification of the Kennedy ('27) method, the dry ashing being carried out at a temperature of 400°C. or less. Color comparison was made with a Cenco-Sheard-Sanford photelometer. The liquid and dry milk samples, as well as the iron compounds tested, were analyzed by the same method.

EXPERIMENTAL RESULTS

On the basis of the experiments of Smith and Otis ('37), it appears that a daily intake of 0.2 mg. of iron by young anemic rats might be an advantageous one to use in testing the relative utilization of iron in ferric phytate, sodium iron pyrophosphate and ferrum reductum as compared with the iron of ferric chloride, since this level permits considerable, but not maximal, regeneration of hemoglobin. However, it seemed advisable to test this level under our conditions of feeding. Hence, four pairs of rats were depleted of iron until the hemoglobin level in the blood averaged 4.56 gm. per 100 cc.

One rat in each pair was then given daily 0.2 mg. of iron as ferric chloride and the other rat 0.3 mg., and the intake of milk was equalized within each pair. At the end of the first, second and third weeks, the average hemoglobin contents of the rats receiving 0.2 and 0.3 mg. of iron daily were, respectively, 9.93 and 11.36 mg., 13.07 and 15.10 mg., and 15.34 and 15.94 mg. These results indicate that a daily dose of 0.2 mg. of iron is insufficient for maximal hemoglobin regeneration in nutritional anemia and, hence, is a suitable dose for the purpose of this experiment. They also reveal the fact that the differences between graded doses of iron in their effects upon hemoglobin regeneration tend to disappear as time goes on. They may be clearly evident at the end of the first week of feeding.

The average results of the hemoglobin regeneration tests, with their statistical evaluations according to the method of Student ('08, '25), are assembled in table 1. It is evident that the iron in sodium iron pyrophosphate, as well as the iron in ferrum reductum, is as well utilized in hemoglobin regeneration as is the iron of ferric chloride. This is as evident after 1 week of feeding as after 3 or 4 weeks. The probabilities favoring a fortuitous outcome of the experiment are entirely too large to be disregarded.

The iron in ferric phytate, however, is definitely inferior to that in ferric chloride in its ability to promote hemoglobin regeneration, a difference that was clearly evident after 1 week of feeding. In estimating the quantitative difference between these two iron compounds, we preferred to use the increments in hemoglobin regeneration observed after 1 week of feeding, because of the tendency, brought out in our initial test with two levels of ferric chloride, for differences to be obscured in the course of time, and also because of the irregularities sometimes displayed by the later hemoglobin determinations that were probably due to variable losses of blood in previous sampling operations and variable recoveries. The average difference in hemoglobin regeneration between pair mates in all eleven pairs of experiment 3 at the end of 1 week

TABLE 1

Hemoglobin regeneration on different iron salts.

TABLE 1

Hemoglobin regeneration on different iron salts.

EXPT. NO.	IRON SALTS COMPARED	AVERAGE LENGTH OF TEST	NO. OF RATS	DAILY INTAKE OF IRON	BODY WEIGHTS		HEMOGLOBIN IN BLOOD			AT END OF FIRST WEEK	
					Average initial weight	Average total gain	Average initial value	Average total gain	Probability of fortuitous outcome	Average gain in hemoglobin	Probability of fortuitous outcome
		days		*mg.*	*gm.*	*gm.*	*gm./100 cc.*	*gm./100 cc.*		*gm./100 cc.*	
1	Ferric chloride	28	7	.2	72	25	5.52	10.17	0.36	3.91 [1]	0.27
	Ferrum reductum	28	7	.2	70	21	5.86	9.81		3.54 [1]	
2	Ferric chloride	21	8	.2	71	23	4.78	9.38	0.46	6.11	0.37
	Sodium iron pyrophosphate	21	8	.2	67	29	4.98	9.53		5.83	
3	Ferric chloride	46	11	.2	92	27	5.91	10.30	0.008	4.10	<0.0001
	Iron phytate	46	11	.2	94	26	5.55	6.66		2.00	
4	Ferric chloride	21	8	.2	80	33	4.47	10.34	0.32	5.79	0.48
	Iron phytate	21	8	.36	80	33	4.60	10.08		5.82	

[1] Averages for eight instead of seven pairs of rats. One rat in one of the pairs died just prior to the termination of the feeding test.

TABLE 2

Iron storage on different iron salts.

EXPT. NO.	IRON SALTS COMPARED	LENGTH OF TEST	NO. OF RATS	ESTIMATED INITIAL CONTENT OF IRON	DETERMINED FINAL CONTENT OF IRON	AVERAGE TOTAL IRON RETAINED	PROBABILITY OF FORTUITOUS OUTCOME	TOTAL IRON INTAKE			PER CENT OF IRON INTAKE RETAINED
								In food	In supplement	Total intake	
		days		*mg.*	*mg.*	*mg.*		*mg.*	*mg.*	*mg.*	
1	Ferric chloride	28	8	1.17	3.99	2.82	.027	.33	5.60	5.93	48
	Ferrum reductum	28	8	1.13	3.69	2.56		.33	5.60	5.93	43
2	Ferric chloride	21	3	.95	3.55	2.55	.087	.31	4.20	4.51	56
	Sodium iron pyrophosphate	21	3	.89	4.18	3.29		.31	4.20	4.51	73
3	Ferric chloride	61	6	1.29	5.32	4.03	.063	.96	12.2	13.16	31
	Iron phytate	61	6	1.25	4.19	2.94		.96	12.2	13.16	22
4	Ferric chloride	21	8	1.17	3.79	2.62	.32	.42	4.20	4.62	57
	Iron phytate	21	8	1.18	3.78	2.60		.42	7.56	7.98	33

of feeding, expressed as a percentage of the hemoglobin increment on ferric chloride, was 56. This calculation indicates that the iron of ferric phytate is only 56%[3] as available for hemoglobin regeneration as the iron of ferric chloride. On the basis of this calculation, a fourth experiment was undertaken with eight pairs of rats comparing 0.2 mg. of iron as ferric chloride with 0.36 mg. of iron as ferric phytate. As the averages in table 1 show, the results of this experiment were quite negative in the sense that no difference was found between the hemoglobin regenerating capacity of 0.2 mg. of iron as ferric chloride and 0.36 mg. of iron as ferric phytate.

The iron retention data presented in table 2 based on carcass analyses of some of the test rats at the end of their feeding period and of litter mate check rats killed at the start of the experiment, bear out the conclusions drawn from the hemoglobin regeneration data. The average percentage iron retentions, computed in the last four columns of the table, are lower the longer the feeding tests and the poorer the availability of the dietary iron. However, they cannot be considered as complete measures of biological availability of the dietary iron, because they do not take into account the replacement of endogenous losses of iron, which might be considered as a maintenance iron requirement, dependent in part upon the continual degradation of erythrocytes. This endogenous replacement seems impossible to compute, even though it were related entirely to erythrocyte destruction (Graam, '42), because of the reutilization of liberated iron to form new hemoglobin (Cruz, Hahn and Bale, '42).

DISCUSSION

The high availability of the iron in sodium iron pyrophosphate and in ferrum reductum testifies to their high value as supplements for the enrichment of white flour. Elvehjem, Hart and Sherman ('33) have reported a high availability of

[3] Although experiment 4 was planned on the basis of this interpretation, it is evident that the iron of ferric phytate, as indicated by the results of experiment 3, is only 44% as available as that of ferric chloride.

the iron in ferric pyrophosphate, although the level of iron fed (0.5 mg. daily) was so high as possibly to obscure some slight inferiority of this form of iron (Smith and Otis, '37). The value of the reduced iron indicates rather clearly, also, that the iron contamination of food materials due to the abrasion of iron-containing grinding mills may contribute to their nutritive value. Such contamination is not inconsiderable. In this laboratory we have found, for example, that whole corn with an original iron content of 0.0035%, will possess an iron content of 0.0061% when ground through a cutting (Wiley) mill, and an iron content of 0.0125% when ground through a burr mill. This added iron is probably metallic in nature and as available biologically as the iron of ferric chloride.

The low biological availability of the iron in ferric phytate contrasts with the apparent high availability of the calcium in calcium phytate (Krieger, Bunkfeldt and Steenbock, '40), and the reported failure of Harrison (Ed. in Lancet, '42) to depress appreciably the utilization by anemic rats of the iron in ferrous carbonate by the addition of sodium phytate. Until the latter experiment is reported in full, no comment can be made on the apparent discrepancy between the results obtained and those secured in this experiment. Probably the considerably higher utilization by adult human subjects of the iron in white bread as compared with 92% extraction bread (Widdowson and McCance, '42) may be explained on the basis of the low availability of ferric phytate, if it may be assumed, as seems reasonable, that phytic acid occurs only in the outer layers of the wheat kernel, and that the iron in patent white flour is largely inorganic in form and originates largely in the milling process. Experiments on the availability of the iron of whole wheat for hemoglobin regeneration in the rat have given strangely discordant results (Free and Bing, '40). In all likelihood this is a penalty for the naive assumption upon which the bioassay methods used are based, to the effect that the only factors determining the rate of hemoglobin regeneration, if all nutrients other than iron essential for hemo-

globin synthesis are placed before the anemic rats, are the amount and the availability of the iron consumed. Some preliminary experiments have been made in this laboratory, in which (a) the intake by paired anemic rats of iron from whole wheat and from ferric chloride was equalized, or otherwise controlled, at sufficiently low levels, (b) adequate amounts of copper and manganese were incorporated in the diets, (c) the intake of milk was equalized within pairs, and (d) the rate of growth of paired rats was kept the same by administering sugar to the rats receiving ferric chloride in order to compensate for the extra calories contained in the wheat given to their pair mates. These experiments, involving ten pairs of rats, indicate that the iron of whole wheat is more than 50% as available for hemoglobin regeneration as the iron of ferric chloride, but not more than 66% as available.

Attempts were made to determine the available iron in sodium iron pyrophosphate, ferrum reductum and ferric phytate by the bipyridine method, following the procedure of Kohler, Elvehjem and Hart ('36). Under the conditions of the test only a small proportion of the iron went into solution, from 2 to 17%, and these proportions bore no close relation to the results of the bioassays. McCance ('39) has published a very friendly discussion of this method of determining the biological availability of iron by chemical means, but he is nevertheless disturbed at the great variation in the results obtained on the same foods in different laboratories, and dubious of the validity of the basic assumption that the non-hematin non-ionizable iron is non-available. The opinion not uncommonly held that the mineral elements of the diet must be in ionizable form to be available to the animal body would seem to be a gratuitous one, and is quite contrary to many observations, among which is the conclusion that calcium phytate is well utilized in bone calcification (Krieger et al., '40), although poorly if at all ionizable (Yang, '40).

SUMMARY AND CONCLUSIONS

The availability of the iron in sodium iron pyrophosphate, ferrum reductum and ferric phytate for hemoglobin regenera-

tion in anemic rats was compared in paired-feeding experiments with that of ferric chloride. The total storage of iron in rats on the various test diets was also determined by carcass analysis. The results of the experiments, involving a total of thirty-five pairs of rats, would seem to justify the following conclusions:

1. When the intake of the basic low-iron diet, as well as the intake of iron in the iron-containing material under test, is equalized among comparative animals, valid comparisons may be obtained after 1 week of feeding.

2. A daily intake of 0.2 mg. of iron by young anemic rats is insufficient for maximum hemoglobin regeneration, but the difference in this respect between doses of 0.2 and 0.3 mg. of iron as ferric chloride becomes less as the feeding period is continued and may practically disappear in 3 weeks' time.

3. The iron of sodium iron pyrophosphate and of ferrum reductum is as well utilized for hemoglobin regeneration as the iron of ferric chloride.

4. The iron of ferric phytate is only about half as well utilized as the iron of ferric chloride.

5. Total iron retentions confirmed the results of hemoglobin regeneration in these comparative tests, though they did not provide as decisive evidence.

6. The low utilization of the iron in ferric phytate may explain the apparently low utilization of the iron of wheat, for which some additional evidence is offered.

LITERATURE CITED

CRUZ, W. O., P. F. HAHN AND W. F. BALE 1942 Hemoglobin radioactive iron liberated by erythrocyte destruction (acetylphenylhydrazine) promptly reutilized to form new hemoglobin. Am. J. Physiol., vol. 135, pp. 595–599.

EDITORIAL 1942 Phytic acid and iron absorption. Lancet, vol. i, p. 595.

ELVEHJEM, C. A., E. B. HART AND W. C. SHERMAN 1933 The availability of iron from different sources for hemoglobin formation. J. Biol. Chem., vol. 103, pp. 61–70.

FREE, A. H., AND F. C. BING 1940 Wheat as a dietary source of iron. J. Nutrition, vol. 19, pp. 449–460.

GRAAM, D. G. 1942 The average length of life of the red corpuscle. J. Lab. Clin. Med., vol. 27, pp. 448–459.

KEMMERER, A. R., C. A. ELVEHJEM, E. B. HART AND J. M. FARGO 1932 The nutritive value and efficiency of mineralized milk. Am. J. Physiol., vol. 102, pp. 319–324.

KENNEDY, R. P. 1927 The quantitative determination of iron in tissues. J. Biol. Chem., vol. 74, pp. 385–391.

KINDER, F., W. S. MUELLER AND H. S. MITCHELL 1942 The availability of the iron of cocoa and of iron-fortified cocoa mixtures. J. Dairy Sci., vol. 25, pp. 401–408.

KOHLER, G. O., C. A. ELVEHJEM AND E. B. HART 1936 Modifications of the bipyridine method for available iron. J. Biol. Chem., vol. 113, pp. 49–53.

KRIEGER, C. H., R. BUNKFELDT AND H. STEENBOCK 1940 Cereals and rickets. XI. Calcium phytate as a source of calcium. J. Nutrition, vol. 20, pp. 15–18.

MCCANCE, R. A. 1939 Ionizable and available iron in foods. Chem. and Industry, vol. 58, pp. 528–530.

MITCHELL, H. H., AND T. S. HAMILTON 1937 Sex differences in anemic rats. Science, vol. 85, pp. 364–366.

ROSE, M. S., AND H. J. HUBBELL 1938 The influence of sex on iron utilization in rats. J. Nutrition, vol. 15, pp. 91–102.

SANFORD, A. H., AND C. SHEARD 1930 The determination of hemoglobin with the photo-electrometer. J. Lab. Clin. Med., vol. 15, pp. 483–489.

SMITH, M. C., AND L. OTIS 1937 Hemoglobin regeneration in anemic rats in relation to iron intake, with suggestions for improvement of the bioassay technic for measuring available iron. J. Nutrition, vol. 13, pp. 573–582.

STUDENT 1908 The probable error of a mean. Biometrika, vol. 6, pp. 1–25.

———— 1925 New tables for testing the significance of observations. Metron, vol. 5, pp. 105–120.

STUGART, R. 1931 Determination of iron in milk and other biological materials. Ind. Eng. Chem., Anal. Ed., vol. 3, pp. 390–393.

TOBEY, J. A., AND W. H. CATHCART 1941 Fortification and restoration in the baking and dairy industries. Ind. Eng. Chem., vol. 33, pp. 714–716.

WIDDOWSON, E. M., AND R. A. MCCANCE 1942 Iron exchanges of adults on white and brown bread diets. Lancet, vol. 242, pp. 588–591.

YANG, E. F. 1940 Ionization of calcium phytate. Nature, vol. 145, p. 745.

FACTORS AFFECTING THE THIAMINE CONTENT OF BREAST MILK [1]

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FOUR FIGURES

(Received for publication July 20, 1942)

The thiamine content of breast milk and the factors affecting it have long been of interest to workers in this laboratory. It has been difficult, however, to obtain fully reliable data on this subject because of the inadequacy of the methods available for the assay of fluids of low thiamine content. It is well-known that the biological method is too cumbersome for routine assays and is unreliable when the test dose is bulky, rich in calories and relatively low in thiamine content. The thiochrome method, which has been used most by recent workers, also presents difficulties in that thiamine may be lost in the precipitation of the proteins and during extraction of the vitamin from permutit. Recovery of added thiamine is more variable than is desired for accuracy, breast milk appearing to offer more complications in this respect than have been observed with thiochrome assays on other biological materials. If the shortcut of treating the milk directly with the oxidizing reagent is tried, there is a tendency for formation of an emulsion which is extremely difficult to break and which therefore leads to low values.

The micro-fermentation technic of Atkin, Schultz and Frey ('39) appears to offer certain advantages. With this method

[1] Assistance in the preparation of these materials was furnished by the personnel of Works Project Administration, Official Project No. 30230.

preliminary extraction of the vitamin is unnecessary. By means of modifications suggested by Schultz,[2] not only the thiamine content, but that of pyrimidine as well can be determined. This latter is important if the thiamine molecule has undergone cleavage. The method is thus practical and useful for vitamin B_1 studies on blood and urine, as well as for milk analyses. A further advantage is found in the extremely small size aliquots required for these assays.

The application of the micro-fermentation procedure to milk thiamine assays is as follows:

The total vitamin B_1 content of milk is measured by introducing the milk directly into the fermentation flasks, without any previous treatment. Usually 0.1 ml. of milk is a satisfactory amount for an aliquot. Water to make a total volume of 1.0 ml., buffer, and salts-sugar-yeast[3] solution are then added and the procedure carried out in accordance with the directions of Atkin et al. ('39).

The pyrimidine content is determined after the milk has been treated with sodium sulfite. Five milliliters of milk are heated for 30 minutes in a boiling water bath with 5 ml. of freshly prepared 4% $Na_2SO_3 \cdot 7H_2O$, and 1 ml. HCl to bring the pH to 2–3. After cooling, excess sulfite is destroyed with 3% hydrogen peroxide, using starch-iodide as an outside indicator; the mixture made neutral to litmus paper with N and N/10 NaOH; and the volume then adjusted to 10 ml. Aliquots of 1.0 ml. of the sulfite-treated milk are used, and the procedure carried through as for untreated milks.

Pyrimidine is calculated as micrograms of thiamine so that the true thiamine content of the milk can be determined readily by the difference between total B_1 and pyrimidine values.

Thiamine in blood is determined by pipetting whole oxalated blood directly into a fermentation flask which already contains the necessary water, and then heating the flask for 2 minutes in a boiling water bath to inactivate certain enzymes of the

[2] Personal communication.

[3] The fresh yeast was supplied twice each week through the courtesy of Standard Brands, Inc., Chicago, Ill.

blood. Flasks are cooled before adding the buffer and salts-sugar-yeast mixture. For most blood samples, 0.3 ml. is a convenient aliquot. Because of the difficulty in preparing a homogenous sample after treating blood with sulfite when micro quantities must be used, we have omitted pyrimidine determinations on blood. Our control tests had shown these values to be very low.

The reliability of the microfermentation procedure was checked by simultaneous assays with thiochrome and biological methods. In addition recovery experiments of added thiamine [4] and standardized dried yeast [5] gave results within $\pm$ 5% of the theoretical. Since our work was started a paper has appeared by Gorham et al. ('42) which includes a thorough discussion of the reliability of the method.

Reports in the literature give thiamine contents in breast milk varying over the wide range of 0 to 32 μg. per cent. Kendall ('42) used the thiochrome method to study milk during the first 9 days after parturition. He reported a range of thiamine from 0 to 9 μg. per 100 ml. of breast milk, with very low values for colostrum and a slow increase in the thiamine content as the true milk appeared. Neuweiler ('38), using the thiochrome method, published values from a trace to 13 μg. per cent, with an average of 10 μg. per 100 ml. of milk. Toverud ('40), likewise using a thiochrome method, at first found a mean value of 4.55 μg. of thiamine for women on an ordinary diet; with improved technic she reported a mean value of 10.8 μg. of thiamine per 100 ml. of breast milk. When supplementary thiamine was given to her women she found mean values of 6.46 and 19.12 μg. when the two types of technics were used. Morgan and Hayes ('39), by means of a biological technic, have studied the breast milk of two women with different nutritive backgrounds. The woman who had the poorer dietary record had breast milk containing 11 μg. per 100 ml. This value was increased to 25 μg. when 14.2 mg. of thiamine were given daily for a period of 1 month. The other

[4] The thiamine was kindly furnished by Merck & Company, Inc.

[5] Standardized dried yeast was provided by the Fleischmann Laboratories.

woman, who was a student of nutrition, had the higher value for her milk of 32 μg. per cent. When given a 10 mg. thiamine supplement, the vitamin in her milk did not increase, but instead decreased slightly and 25 μg. were found per 100 ml. Apparently her regular diet furnished adequate thiamine to meet the capacity of her mammary glands. Earlier studies in our own laboratory (Knott, '42) for which both biological and thiochrome technics were employed, gave values comparable to those of both Toverud, and Morgan and Hayes for their women on ordinary diets. Our range of thiamine for seventeen women was from 3 to 18 μg. per 100 ml. of breast milk with an average of 9 μg. per cent.

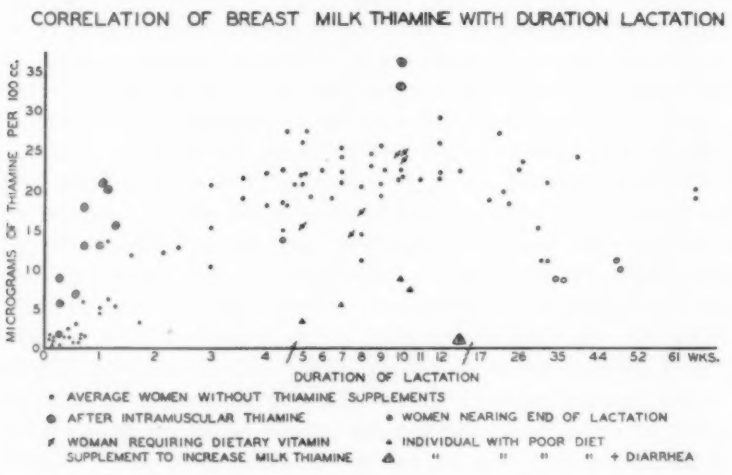
CORRELATION OF BREAST MILK THIAMINE WITH DURATION LACTATION
MICROGRAMS OF THIAMINE PER 100 cc.
0
5
10
15
20
25
30
35
1 2 3 4 5 6 7 8 9 10 11 12 17 26 35 44 52 61 WKS.
DURATION OF LACTATION
• AVERAGE WOMEN WITHOUT THIAMINE SUPPLEMENTS
● AFTER INTRAMUSCULAR THIAMINE
⊕ WOMEN NEARING END OF LACTATION
WOMAN REQUIRING DIETARY VITAMIN SUPPLEMENT TO INCREASE MILK THIAMINE
▲ INDIVIDUAL WITH POOR DIET
△ " " " " + DIARRHEA

Figure 1

During the past year we have used the micro-fermentation method to analyze 111 samples of milk from fifty different women in all stages of lactation. The total vitamin B_1 values for all of these samples are summarized in figure 1. During the neonatal period it was frequently difficult to get a large enough sample of milk to analyze both total vitamin B_1 and pyrimidine content. For the sake of comparison all the values

in this figure are for total vitamin B_1. The pyrimidine content of the majority of these milk samples was between 1 and 2 μg. per 100 ml. of milk, so the true thiamine values were only slightly lower than the results graphically presented. The average true thiamine content of all of these samples was 15.1 μg. per 100 ml. of milk.

It will be observed that during the first days after parturition the colostrum milk from average women contained but little thiamine. These results thus verified the report of Kendall. As the true milk began to be secreted, the thiamine content gradually was increased, but at the end of 9 days, only one woman had a level higher than 6 μg. of thiamine per 100 ml. of milk. The details for the individual women during this neonatal period are discussed more fully later. Most of the women appeared to reach their capacity in thiamine content of breast milk at about 3 weeks after parturition. During the remainder of the lactating period values were usually between 18 and 27 μg. of thiamine per 100 ml. of milk. Toward the end of lactation the thiamine content of the milk seemed to decrease. In two cases, where the women were weaning their infants, values of 8.7 and 10.8 were found. There was also a tendency for lower values to be found with women who were having difficulty nursing their infants. The average thiamine content in eighteen samples of breast milk from women whose infants received supplementary feedings was 9.3 μg., as compared to the average of 20.1 μg. for fifty-five analyses with thirty-three women who were more than 20 days post partum and who were raising their infants on unsupplemented breast milk. The adequacy of this breast milk in meeting infant requirements for thiamine is discussed elsewhere (Knott, Kleiger and Schultz, '43).

The blood thiamine values of thirty-seven of our women averaged 5.01 μg. per 100 ml. for sixty-one determinations. In fifteen nonlactating adults we had found an average of 6.16 μg. of thiamine. In figure 2 all of our milk thiamine values are plotted in order of magnitude, with the corresponding blood thiamine levels for each woman ranged beneath. Because we

did not wish to interfere with the daily living regimes of our women, no attempt was made to secure only fasting blood samples. There has been some variation in our results, therefore, due to post absorptive states. Nearly all samples were taken at either 8:00 or 10:30 A.M. It will be seen from figure 2 that, with the exception of one woman's values, there was a definite tendency for low milk values to be associated with low blood thiamine levels and higher milk thiamine with higher blood thiamine. The one obvious deviant with high blood but low milk thiamine is an individual with apparently poor mammary function who will be discussed in detail later.

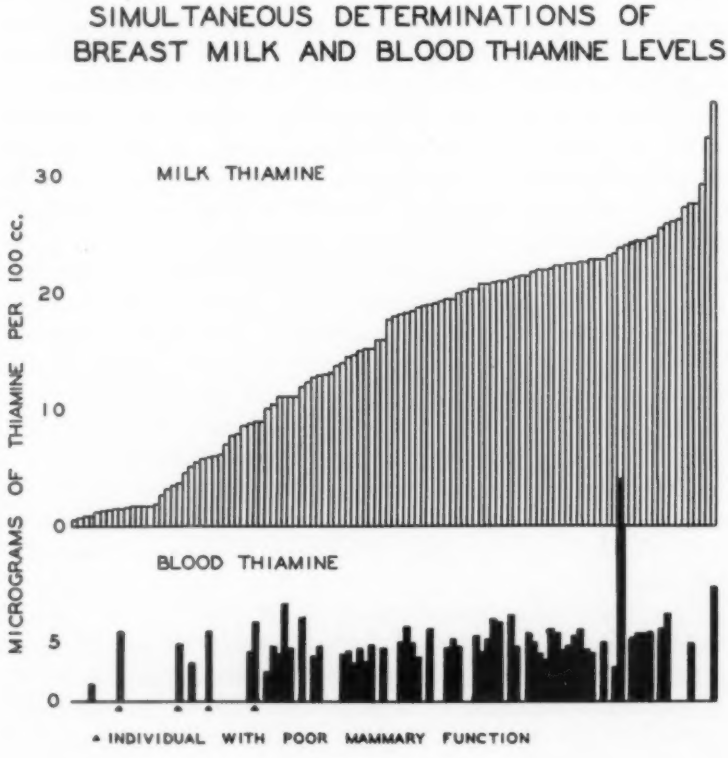

Figure 2

In figure 3 results are summarized for the thirteen women who were studied during the neonatal period. Ten of these women had received routine hospital care, and figure 3 shows a gradual increase in the thiamine content of their milk during the first 9 days of lactation. The other three women had been given 10 mg. of thiamine intramuscularly twice during labor, and 15 mg. of thiamine daily thereafter in divided oral doses with each meal. Their milk values were in marked contrast to the low values of the ten women on routine hospital care. The three with supplementary thiamine had rapid increases in the

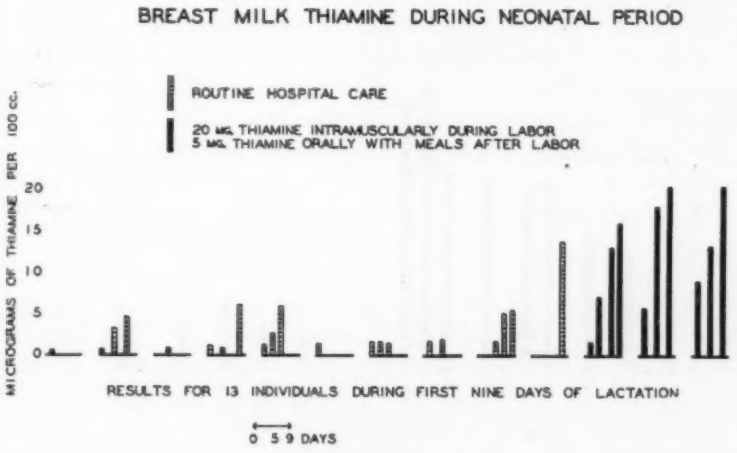
BREAST MILK THIAMINE DURING NEONATAL PERIOD
ROUTINE HOSPITAL CARE
20 MG. THIAMINE INTRAMUSCULARLY DURING LABOR
5 MG. THIAMINE ORALLY WITH MEALS AFTER LABOR
MICROGRAMS OF THIAMINE PER 100 CC.
20
15
10
5
0
RESULTS FOR 13 INDIVIDUALS DURING FIRST NINE DAYS OF LACTATION
0 5 9 DAYS

Figure 3

thiamine content of their milk, until at the end of 9 days it was as high as most women achieved in 3 or 4 weeks. The trend in results for these women has been remarkably consistent. Since it is supported by additional unpublished data, it leads to the conclusion that the average woman undergoes a temporary thiamine deficiency due to the reduced food intakes during parturition, and to the ordeal of labor. About 3 weeks are apparently necessary under ordinary conditions for the depleted thiamine stores of the body to be rebuilt. The use of sup-

plementary thiamine would seem to accomplish this restoration in 1 week.

Figure 4 shows the progress of two women who were low in both breast milk and blood thiamine at the time of their first analyses 5 weeks after the start of lactation. Mrs. H. was able to raise her milk and blood thiamine levels by regular use of wheat germ as a daily dietary supplement. The thiamine content of her milk increased from 15.9 and 14.7 μg. to 24 μg.

THE EFFECT OF SUPPLEMENTARY VITAMIN UPON BLOOD AND BREAST MILK THIAMINE LEVELS OF TWO WOMEN

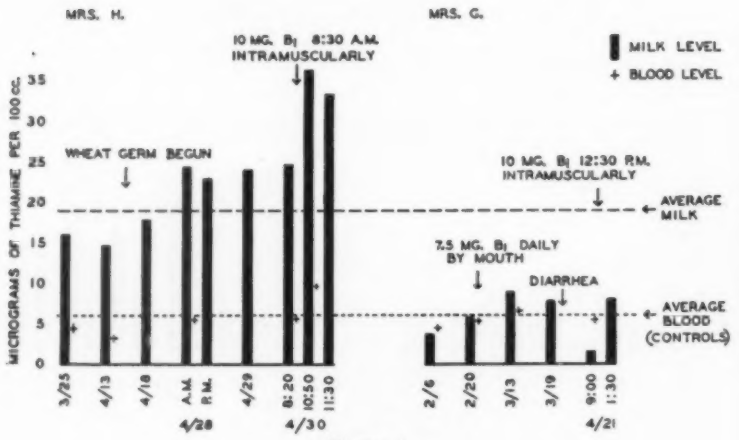

Figure 4

per 100 ml. She maintained this high level consistently for four measurements taken during 3 consecutive days. It is interesting that her milk reached the still higher level of 36.2 μg. within 2 hours after 10 mg. of thiamine were injected intramuscularly. This last high level was only transient, however, for at the end of another hour the milk had decreased in thiamine content to 33.2 μg. per 100 ml. Mrs. G.'s case was much more complex. She had the exceptionally low milk thiamine content of 3.7 μg. at her first analysis. Neither 2.5 mg. of thiamine as a dietary supplement to each meal, nor special

dietary instruction, brought her milk thiamine content higher than 9.0 μg. per cent. A chronic diarrhea of 2 weeks' duration, occurring during a period of careless food habits, brought her milk thiamine down to only 1.5 μg. The level of her blood thiamine, however, had increased above its initial level and remained at satisfactory levels after therapy was started.

These two women would seem to illustrate two types of response. Both women undoubtedly were deficient in thiamine initially, but Mrs. H. responded more efficiently to the use of supplements. The conclusion seems justified that Mrs. H.'s low milk thiamine had been due to an inadequacy of diet in meeting her specific needs, while Mrs. G. may have had a mammary inefficiency complicating her dietary deficiency. It must be mentioned, however, that Mrs. G. had a much poorer background, both economically and in regard to training in adequate food habits. Her poor mammary function therefore may have been the result of long-continued sub-optimal intakes and the present study was inadequate in meeting her needs.

Careful dietary records were kept for 4 or more days by most of the women who were studied after the neonatal period. When these records were analyzed for thiamine intakes, it was found that many women had been able to secrete milk containing 20 or more μg. of thiamine per 100 ml. while their food intakes were contributing about 1.5 mg. of thiamine daily. Other women on this level of intake had milk of lower thiamine content. A safer intake to insure good mammary function would probably be about 2 mg. of thiamine daily. It was apparent, however, that individual needs varied greatly. Specific illustrative cases were encountered to show that the total quantity, as well as the quality of the food, the past dietary history of the mother, the degree of nervous tension, the amount of activity, and the occurrence of diarrhea, all were factors in addition to thiamine intakes which affected results.

SUMMARY

1. Thiamine has been determined by micro-fermentation procedure in 111 samples of breast milk from fifty different women.

2. The average thiamine content of all samples was 15.1 μg. per 100 ml. of milk. Women who were nursing their infants without using supplementary formulas had milk containing 20.1 μg. of thiamine per 100 ml. Women who had to use supplementary feedings for their infants, or who were weaning their infants, had an average thiamine content of 9.3 μg. per cent for their milk.

3. Colostrum contained almost no thiamine and the vitamin increased only gradually in the milk as lactation progressed. Most women required 3 or more weeks before their milk reached the level of thiamine content characteristic for the individual. The use of intramuscular thiamine during labor, and of oral thiamine supplements post partum, caused a rapid increase in the thiamine content of the milk within 9 days to values such as most women achieved in 3 or 4 weeks.

4. There was a tendency for low milk thiamine values to be associated with low blood thiamine values, and higher milk levels with higher blood levels.

5. Some women could secrete milk containing 20 μg. or more per 100 ml. on intakes of about 1.5 mg. of thiamine. Other women required higher dietary thiamine or thiamine therapy.

LITERATURE CITED

ATKIN, L., A. S. SCHULTZ AND C. N. FREY 1939 Ultramicro-determination of thiamin by the fermentation method. J. Biol. Chem., vol. 129, p. 471.

GORHAM, A. T., J. C. ABELS, A. L. ROBINS AND C. P. RHOADS 1942 The measurement and metabolism of thiamin and of a pyrimidine stimulating yeast fermentation found in the blood cells and urine of normal individuals. J. Clin. Invest., vol. 21, p. 161.

KENDALL, N. 1942 Thiamin content of various milks. J. Pediat., vol. 20, p. 65.

KNOTT, E. M. 1942 The thiamin content of milk in relation to the vitamin B_1 requirement of infants. Am. J. Pub. Health., vol. 32, p. 1013.

KNOTT, E. M., S. C. KLEIGER AND F. W. SCHULTZ 1943 Is breast milk adequate in meeting the thiamin requirement of infants? J. of Pediatrics. (In press.)

MORGAN, A. F., AND E. G. HAYES 1939 Vitamin B_1 content of human milk as affected by ingestion of thiamin chloride. J. Nutrition, vol. 18, p. 105.

NEUWEILER, W. 1938 Über den gehalt der Frauenmilch an vitamin B_1 und seine Beeinflussbarkeit durch die ernährung. Klin. Wchnschr., Bd. 17, S. 296.

TOVERUD, K. U. 1940 The excretion of aneurin in pregnant and lactating women and in infants. Z. Vitamin forsh., vol. 10, p. 255.

DISAPPEARANCE OF CELLULOSE AND HEMICELLULOSE FROM THE DIGESTIVE TRACTS OF CHILDREN [1]

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ONE FIGURE

(Received for publication August 7, 1942)

The question of digestion and utilization of fiber has been studied for many years, but since the problem is of particular importance in assessing the nutritive value of animal feeds, most of which contain large amounts of fiber, animals have been used in the majority of the experiments. The need for further knowledge of the fate of complex carbohydrates in their transit through the alimentary tract of man has been emphasized by: (a) the recent campaign for nationwide use of higher extracted flours (Sealock, Basinski and Murlin, '41); (b) consideration of the special physiologic value of indigestible carbohydrate in the diet of man (Cowgill and Anderson, '32; Cowgill and Sullivan, '33) and of children (Hunscher, Hummel and Macy, '40); (c) investigation of their bacteriocidal and bacteriostatic activity on intestinal pathogens (Werch, Jung, Plenk, Day and Ivy, '42) and (d) study of the type of micro-organisms in human feces that are responsible for cellulose splitting during passage through the intestinal canal (Weiss and Rettger, '37; Hirschberg, '42). The progress of research in this field was reviewed by Mangold ('34) and apparently the fact is estab-

[1] A report of this work was presented before the meeting of the American Institute of Nutrition, at New Orleans, on March 13, 1940.

lished that cellulose and hemicellulose may be broken down to fatty acids and gases by the action of symbiotic microorganisms in the intestine.

Although the presence of glucose as an intermediate product in the fermentation of cellulose has been questioned recently (Woodman and Evans, '38), the end-products of cellulose digestion in man never have been shown, conclusively, to provide nourishment. Most of the evidence recorded in the literature is based on single experiments with a limited number of subjects; workers have been hampered by a lack of suitable analytical methods (Crampton and Maynard, '38) and by the extremely wide range of variation in individual subjects (Sealock et al., '41). It has been impossible to compare the results obtained in different laboratories, or to formulate definite conclusions regarding the disappearance of these "unavailable carbohydrates" in the intestinal tracts. McCance and Lawrence ('29), after reviewing the literature, reached the conclusion that the digestion of the complex polysaccharides in man yields products useful only to the bacteria which bring it about, and called these carbohydrates "unavailable," and unimportant in energy value from the standpoint of human nutrition.

Although the complex carbohydrates cannot be demonstrated to furnish energy, they undoubtedly have a definite role in the diet of man, from the standpoint of laxation (Cowgill and Anderson, '32; Cowgill and Sullivan, '33). The mode of action of these substances in increasing stool volume is not known; it seems unlikely that they act by virtue of bulk alone, since they can only increase the weight of feces by 2% (Manville, Bradway and McMinis, '36) and the amount of water which they can absorb is limited because they are insoluble. Williams and Olmsted ('36) suggest that it is the amount of cellulose and hemicellulose which disappears during passage through the intestine which influences the volume of feces, and that there also appears to be a relationship between this disappearance and the amount of volatile fatty acid excreted.

Most, if not all, of the experiments on the utilization or laxation value of the "unavailable carbohydrates" have been carried out on adult male subjects, and for a limited period of time. It has been suggested (McCance and Lawrence, '29) that a study of women and children might yield somewhat different results; experiments of longer duration and the correlation of individual utilization of crude fiber with physiological type, habits of eating and dentition, have long been needed (Mangold, '34). The present investigation fulfills these requisites of study and contributes pertinent information on the disappearance of cellulose and hemicellulose from food as it passes through the digestive tracts of children. A discussion of the results in relation to other biochemical data obtained with the same children is contained in another paper (Macy, Hummel and Shepherd, '42).

EXPERIMENTAL

Organization of study. During a study of nutrition and chemical growth with average, healthy, pre-adolescent children (Macy, '42), data were procured on the lignin, cellulose and hemicellulose present in the diet and excreted in the feces. Eleven children in one group were studied continuously for periods up to 225 consecutive days; seven children in another group were studied for 55 consecutive days. The gastrointestinal patterns of the subjects were known (Macy, Reynolds, Souders and Olson, '40; Reynolds, Macy, Hunscher and Olson, '40); the lipid and mineral distributions in the feces were available; and the fact that the children were maintained on approximately constant diets of common foods [2] provided an excellent opportunity for studying the individual variations in the disappearance of cellulose and hemicellulose from the intestinal tract. The subjects' eating habits were good, their bowel movements were regular and no cathartics were necessary throughout the study.

[2] The foods used in the diets were: corn flakes, white and whole wheat bread, graham crackers, apple, banana, orange and tomato juice, cabbage, carrots, lettuce, peas, potato, peanut butter, lean beef, eggs, cheese, gelatin, butter, honey, sugar and salt.

Preparation of material. The fecal composites for the 5-day balance periods were separated by means of carmine markers taken with breakfast on the first day of each period. After collection and separation of the stools, the material in each composite was thoroughly mixed and an aliquot dried to constant weight in a steam oven at 60°C., with alcohol to suppress bacterial decomposition. Foods were oven-dried in vacuo at 40°C. Each sample was analyzed in triplicate by the method of Williams and Olmsted ('35), for lignin, cellulose and hemicellulose. Other publications from this laboratory have included the details of sampling, collection and analysis (Macy, '42), and discussion of desiccation methods (Teague, Galbraith, Hummel, Williams and Macy, '43).

RESULTS AND DISCUSSION

Table 1 contains the ages, lengths, weights, laxation rates, fiber intakes and the average daily wet and dry weights and total fiber contents of the feces of the children. These data demonstrate wide variations in the response of individuals receiving similar intakes of fiber, and show the increased disappearance of fiber in the older, larger children, accompanied by a generally decreased laxation rate. It is obvious that, notwithstanding the augmented fiber intake of the older subjects in group II (subjects XII–XVIII), lower percentages of the intakes were found in the feces and represented considerably smaller fractions of the dry weights of the feces. Since lignin is not broken down in the alimentary canal the increased disappearance of complex carbohydrates from the digestive tracts of the older children must have resulted from fermentation of cellulose and hemicellulose.

Tables 2 and 3 present the range and the mean of 24-hour values for the excretion and disappearance of cellulose, total hemicellulose and stable hemicellulose from the intestinal tracts. The average daily excretion of cellulose in the feces (table 2) ranged from 0.03 to 2.26 gm., which represents a disappearance during passage through the intestinal tract of from 2.6 to 98.5% of the cellulose intake. The mean values

for the individuals, over the entire time each was studied, ranged from 0.42 to 1.04 gm. per day for the amount excreted in the feces, and 1.21 to 2.46 gm. for that which disappeared. The difference in fecal cellulose displayed by individuals (maximum minus minimum values) ranged from 0.25 gm. for subject XVI to 1.95 gm. for subject II, indicating the response

TABLE 1

Average daily intake and excretion of complex carbohydrates by eighteen children.

SUBJECT	OBSERVED	AGE	RECUMBENT LENGTH	BODY WEIGHT	PHYSICAL TYPE [1]	LAXATION RATE [2]	FIBER INTAKE [3]	FECAL WEIGHT		FECAL FIBER [3]		
								Wet	Dry	Total	Of dry feces	Of fiber intake
	days	*yrs.*	*cm.*	*kg.*				*gm.*	*gm.*	*gm.*	*%*	*%*
I	215	4	108.6	17.99	.0241	2.0	5.96	83.9	15.5	3.10	20	52
II	205	5	116.0	19.16	.0231	2.5	5.99	141.3	21.4	4.33	20	72
III [4]	225	5	110.9	19.07	.0242	1.7	5.98	101.2	16.8	3.59	21	60
IV [4]	180	5	106.8	17.68	.0244	1.8	5.55	85.9	14.8	3.08	21	55
V [4]	180	6	118.6	22.12	.0237	1.1	6.35	92.2	16.9	3.58	21	56
VI [4]	215	6	117.1	21.12	.0236	1.3	5.98	82.9	14.6	2.83	19	47
VII	220	6	120.5	22.62	.0235	1.5	6.50	85.5	16.3	3.36	21	52
VIII	210	6	120.8	21.24	.0229	1.7	6.50	76.4	16.1	3.16	20	49
IX	165	8	120.4	24.32	.0240	1.7	6.15	143.6	20.6	3.52	17	57
X	30	8	124.4	23.50	.0230	1.5	6.12	75.2	12.5	2.91	23	48
XI	225	8	138.0	28.14	.0221	1.4	6.33	73.0	17.3	3.03	18	48
XII	55	8	132.2	27.08	.0228	1.4	6.79	78.0	17.7	2.56	14	38
XIII	55	9	140.9	29.04	.0218	1.9	6.79	112.4	22.0	2.70	12	40
XIV [4]	55	9	131.2	28.13	.0232	1.5	6.79	97.5	18.7	3.10	16	46
XV	55	10	144.9	33.64	.0222	1.1	7.01	72.6	18.5	2.15	12	31
XVI	55	10	139.7	31.80	.0226	1.2	7.01	81.0	18.8	2.69	14	38
XVII	55	11	138.3	35.75	.0238	1.1	7.01	116.7	21.2	2.71	13	39
XVIII	55	12	159.1	41.84	.0218	0.8	7.01	77.1	19.8	2.58	13	37

[1] Cube root of weight divided by recumbent length.

[2] Defecations per day.

[3] Cellulose, hemicellulose and lignin.

[4] Girls.

of individuals from time to time to vary as much as the 0.62 gm. difference observed between the average values for individuals. The disappearance of cellulose displayed differences within individuals ranging from 0.25 gm. for subject XVI to 1.83 gm. for subject IV, compared to a range of 1.25 gm. between the means for individuals. This wide range is some-

what contrary to the opinion expressed by Mangold ('34) that "variability of results for fiber digestion is probably also affected by the nature of the rest of the diet fed," for some of these children were maintained on an approximately constant diet composed of the same foods for a period of almost 8 months. The values, however, confirm the statement of

TABLE 2

Average daily fecal excretion and disappearance of cellulose from the alimentary tracts of eighteen children.

SUBJECT	CELLULOSE								
	In feces			DISAPPEARANCE					
				Total			Of intake		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	%	%	%
I	0.05	1.06	0.50	1.26	2.21	1.75	54.3	97.6	78.3
II	0.03	1.98	1.04	0.29	1.99	1.21	12.8	98.5	54.2
III	0.16	1.03	0.51	1.12	2.21	1.57	53.8	93.2	75.5
IV	0.55	1.93	1.04	0.16	1.99	1.31	7.7	78.0	55.2
V	0.19	0.76	0.48	1.54	2.51	1.95	70.3	90.9	80.3
VI	0.19	1.28	0.62	1.25	2.65	1.80	50.0	93.3	74.2
VII	0.45	1.60	0.90	0.94	2.18	1.47	37.5	82.4	61.6
VIII	0.28	1.10	0.65	1.18	2.22	1.71	52.6	88.8	72.2
IX	0.44	2.26	0.98	0.06	1.88	1.34	2.6	81.0	57.8
X	0.40	1.52	0.98	0.80	1.92	1.36	34.5	82.8	58.3
XI	0.23	0.79	0.48	1.53	2.34	1.92	66.0	90.1	79.9
XII	0.36	0.83	0.71	1.91	2.38	2.03	69.7	86.9	74.1
XIII	0.60	0.92	0.73	1.82	2.14	2.01	66.4	78.1	73.4
XIV	0.69	1.53	0.97	1.21	2.05	1.77	44.2	74.8	64.6
XV	0.41	0.90	0.67	1.98	2.47	2.21	68.8	85.8	76.7
XVI	0.27	0.52	0.42	2.36	2.61	2.46	81.9	90.6	85.4
XVII	0.43	1.02	0.75	1.86	2.45	2.13	64.6	85.1	74.0
XVIII	0.36	0.68	0.56	2.20	2.52	2.32	76.4	87.5	80.6

Mangold that "the digestibility of fiber in man is extremely variable, and consequently the experimental results of different workers . . . . can scarcely be compared and used to give averages valid for the general case." It is not surprising that results from different laboratories show variation when individual children show such widely different results, even

TABLE 3

Average daily disappearance of total and stable hemicellulose from the alimentary tracts of eighteen children.

SUBJECT	TOTAL HEMICELLULOSE IN FECES (GM.)			DISAPPEARANCE OF TOTAL HEMICELLULOSE						DISAPPEARANCE OF STABLE HEMICELLULOSE					
				Total (gm.)			Per cent of intake			Total (gm.)			Per cent of intake		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
I	0.36	1.28	0.77	1.02	1.92	1.44	48.2	80.2	65.4	0.61	1.29	0.92	32.3	74.8	54.8
II	0.62	2.24	1.14	0.58	1.65	1.09	26.7	71.4	49.2	0.13	1.10	0.60	7.6	64.0	35.2
III	0.43	1.81	0.90	0.36	1.53	1.10	16.6	75.6	55.3	0.12	1.08	0.70	7.0	68.4	44.8
IV	0.61	1.70	1.08	0.77	1.74	1.25	31.2	71.8	53.7	0.19	1.16	0.72	10.0	62.6	40.0
V	0.56	1.52	1.00	0.95	1.91	1.51	31.5	77.3	60.1	0.37	1.33	0.93	19.6	70.4	48.3
VI	0.52	1.39	0.97	0.78	2.10	1.54	35.9	80.2	61.4	0.33	1.50	0.97	19.2	74.3	49.9
VII	0.50	1.34	1.00	0.83	1.90	1.45	38.2	77.0	59.2	0.38	1.30	0.90	22.1	71.0	47.4
VIII	0.40	1.42	0.83	1.12	1.97	1.51	51.4	81.6	64.6	0.52	1.39	0.97	28.3	76.7	54.1
IX	0.52	1.16	0.80	1.31	1.95	1.67	53.0	79.0	67.6	0.73	1.37	1.09	38.6	72.5	57.7
X	0.63	1.52	1.02	0.95	1.84	1.45	38.5	74.5	58.8	0.37	1.26	0.87	19.6	66.7	46.0
XI	0.45	1.26	0.89	1.21	1.99	1.62	49.0	81.6	64.6	0.63	1.38	1.03	33.3	75.3	53.7
XII	0.54	0.74	0.64	2.06	2.26	2.16	73.6	80.7	77.1	0.87	1.07	0.97	54.0	66.4	60.2
XIII	0.57	0.78	0.68	2.02	2.23	2.12	72.1	79.6	75.7	0.83	1.04	0.93	51.6	64.6	58.0
XIV	0.55	1.03	0.74	1.77	2.25	2.06	63.2	80.4	73.6	0.58	1.06	0.87	36.0	65.8	54.1
XV	0.53	0.71	0.64	2.16	2.34	2.23	75.3	81.5	77.7	0.94	1.12	1.01	57.0	67.9	61.3
XVI	0.51	0.69	0.58	2.18	2.36	2.29	76.0	82.2	79.8	0.96	1.14	1.07	58.2	69.1	65.1
XVII	0.58	0.94	0.69	1.93	2.29	2.18	67.2	79.8	76.0	0.71	1.07	0.96	43.0	64.8	58.3
XVIII	0.49	0.71	0.60	2.16	2.38	2.27	75.3	82.9	79.1	0.94	1.16	1.05	57.0	70.3	63.8

when maintained on a uniform diet and a standardized technique of analysis is used.

The amount of hemicellulose excreted in the feces (table 3) varied from 0.36 to 2.24 gm. per day, a disappearance of from 16.6 to 82.9% of the intake. Since Williams and Olmsted ('36) found that a fraction of the hemicellulose of foods should be classed as "labile," an examination of the data to show whether the "stable" hemicellulose was broken down by intestinal organisms was of interest. Disappearance of the total hemicellulose is determined as the difference between analyses of food intake and feces. Since all of the hemicellulose found in the feces is considered "stable" hemicellulose, the disappearance of the stable fraction is calculated by subtracting the fecal hemicellulose from the analytical values for stable hemicellulose in the diet. The disappearance of stable hemicellulose ranged from 0.12 to 1.50 gm. per day for the eighteen children, or 7.0 to 76.7% of the daily intake. The excretion and disappearance values for hemicellulose and stable hemicellulose also show variations that are as great for the same individuals from time to time as they are between individuals. Fecal hemicellulose varied from 0.18 gm. for subjects XV and XVI to 1.62 gm. for subject II, whereas the averages for individuals showed a range of 0.56 gm.

In the data included in tables 2 and 3 there are evident no definite relationships between excretion or disappearance of cellulose and hemicellulose and sex, intake or age. However, the absolute values for the disappearance of cellulose and hemicellulose and the per cent of hemicellulose intake disappearing tend to be greater for the older group of children. Disappearance of total hemicellulose ranged from 0.18 gm. for subjects XV and XVI to 1.32 gm. for subject VI, as compared to 1.20 between individuals. The disappearance of stable hemicellulose varied from 0.18 gm. for subjects XV and XVI to 1.17 gm. for subject VI, as compared to 0.49 gm. between individuals.

In table 4 the values for cellulose and total hemicellulose in the feces and disappearing in the digestive tracts have

been calculated per kilogram of body weight and arranged according to the weights of the children. The data do not demonstrate parallel relationships with body weight but they do emphasize the variations in the ability of individual children to break down the complex carbohydrates. The results are in keeping, therefore, with our knowledge of the great

TABLE 4

Average daily cellulose and hemicellulose in the feces and disappearing from the gastrointestinal tracts of eighteen children.

(Milligrams per kilogram of body weight.)

SUBJECT	BODY WEIGHT	CELLULOSE		HEMICELLULOSE		CELLULOSE + HEMICELLULOSE	
		In feces	Disappearance	In feces	Disappearance	In feces	Disappearance
IV [1]	17.68	54	69	57	66	111	135
I	17.99	28	97	42	81	70	178
III [1]	19.07	29	89	51	61	80	150
II	19.16	54	63	59	58	113	121
VI [1]	21.12	27	80	43	68	70	148
VIII	21.24	31	80	39	72	70	152
V [1]	22.12	23	92	47	71	70	163
VII	22.62	41	66	46	66	87	132
X	23.50	40	56	42	60	82	116
IX	24.32	42	57	33	71	75	128
XII	27.08	26	75	24	80	50	155
XIV [1]	28.13	35	62	26	74	61	136
XI	28.14	17	68	32	58	49	126
XIII	29.04	25	69	23	73	48	142
XVI	31.80	21	70	20	70	41	140
XV	33.64	13	73	17	68	30	141
XVII	35.75	21	60	19	61	40	121
XVIII	41.84	14	56	14	54	28	110

[1] Girls.

differences in the intestinal flora and the variety of bacteria and molds that break down the complex carbohydrates in the colon of man (Hirschberg, '42; Weiss and Rettger, '37).

The daily intake per child of cellulose plus hemicellulose ranged from 138 to 248 mg. per kilogram of body weight, amounts which, exclusive of the lignin in the diets, are greater than the physiologic roughage minimum of 90 to 100 mg. per

kilogram of body weight proposed by Cowgill and Anderson ('32) for adult men. The daily intake recommended for adults by these investigators was from 6.3 to 7.0 gm. of fiber per day, whereas in this study (table 1) the children's consumption of complex carbohydrates ranged from 5.6 to 7.0 gm. per day, and from 168 to 331 mg. per kilogram of body weight per day.

The average daily excretions of cellulose plus hemicellulose per kilogram of body weight have been plotted against their corresponding intakes and against laxation rate (fig. 1). For

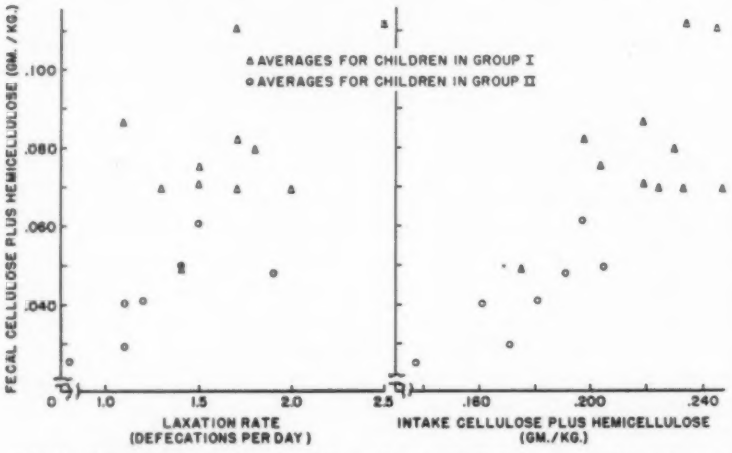

Fig. 1 Relationship of fecal cellulose plus hemicellulose to laxation rate and to intake of cellulose plus hemicellulose.

the children in both groups the scatter diagrams show positive relationships between intake and fecal excretion and between fecal excretion and laxation rate, indicating that on the body weight basis, increased intakes of cellulose plus hemicellulose were accompanied by increased fecal excretion of those substances and increased fecal excretion by higher laxation rates. These relationships, with the apparent increased ability of the older children to decompose cellulose and hemicellulose, may be the result of growth in length of the intestines, pro-

viding greater time for bacterial action. The wide range of individual variation may be due to differences in the fineness of the particles exposed to digestion, peristaltic action, or other physiologic characteristics.

SUMMARY

Eighteen children, ages 4 to 12 years, were observed for from 30 to 225 successive days, during which they consumed uniform diets of known nutritive quality. With an average daily intake per child of 4 to 6 gm. of cellulose plus hemicellulose (138 to 248 mg. per kilogram of body weight) there was satisfactory bowel elimination and all children enjoyed buoyant health throughout the studies.

The average daily fecal excretion of cellulose and hemicellulose for 2455 days ranged from 0.03 to 2.26 gm. (1.5 to 97.4% of the intake) and 0.36 to 2.24 gm. (17.1 to 83.4% of intake), respectively. From 2.6 to 98.5% of the cellulose intake and 16.6 to 82.9% of the hemicellulose were digested by intestinal organisms during passage through the alimentary canal.

From the observations recorded in this study a roughage intake of 5 to 7 gm. of fiber per day, or 168 to 331 mg. per kilogram of body weight per day, provided adequate amounts of complex carbohydrates for normal laxation and all other known physiologic processes of normal children, and there was no evidence of any untoward effects upon the absorption of nitrogen and the mineral elements.

LITERATURE CITED

COWGILL, GEORGE R., AND WILLIAM E. ANDERSON 1932 Laxative effects of wheat bran and "washed bran" in healthy men. A comparative study. J. A. M. A., vol. 98, pp. 1866–1875.

COWGILL, GEORGE R., AND ALBERT J. SULLIVAN 1933 Further studies on the use of wheat bran as a laxative. Observations on patients. J. A. M. A., vol. 100, pp. 795–802.

CRAMPTON, E. W., AND L. A. MAYNARD 1938 The relation of cellulose and lignin content to the nutritive value of animal feeds. J. Nutrition, vol. 15, pp. 383–395.

HIRSCHBERG, NELL 1942 Cellulose splitting micro-organisms in human feces. Am. J. Dig. Dis., vol. 9, pp. 200–202.

HUNSCHER, HELEN A., FRANCES COPE HUMMEL AND ICIE G. MACY 1940 Effect of varied banana intake on nitrogen and mineral balances of normal children. Am. J. Dis. Child., vol. 60, pp. 509–517.

MCCANCE, R. A., AND R. D. LAWRENCE 1929 The carbohydrate content of foods. Med. Research Council, Spec. Rep. Ser. No. 135.

MACY, ICIE G. 1942 Nutrition and Chemical Growth in Childhood. Vol. I. Evaluation. Charles C. Thomas, publisher, Springfield, Illinois.

MACY, ICIE G., FRANCES COPE HUMMEL AND MARION L. SHEPHERD 1942 The value of complex carbohydrates in the diets of children. Am. J. Dis. Child. (In press.)

MACY, ICIE G., LAWRENCE REYNOLDS, HELEN J. SOUDERS AND MARY BATES OLSON 1940 Normal variation in the gastrointestinal response of healthy children. Am. J. Roentgen. Rad. Therap., vol. 43, pp. 394–403.

MANGOLD, ERNST 1934 The digestion and utilisation of crude fibre. Nutr. Abst. Rev., vol. 3, pp. 647–656.

MANVILLE, IRA A., ELIZABETH M. BRADWAY AND AVOCA S. MCMINIS 1936 Pectin as a detoxication mechanism. Am. J. Dig. Dis. Nutr., vol. 3, pp. 570–572.

REYNOLDS, LAWRENCE, ICIE G. MACY, HELEN A. HUNSCHER AND MARY BATES OLSON 1940 The gastrointestinal response of average, healthy children to test meals of barium in milk, cream, meat and carbohydrate media. Am. J. Roentgen. Rad. Therap., vol. 43, pp. 517–532.

SEALOCK, ROBERT R., DANIEL H. BASINSKI AND JOHN R. MURLIN 1941 Apparent digestibility of carbohydrates, fats, and "indigestible residue" in whole wheat and white breads. J. Nutrition, vol. 22, pp. 589–596.

TEAGUE, D. MAXWELL, HARRY GALBRAITH, FRANCES COPE HUMMEL, HAROLD H. WILLIAMS AND ICIE G. MACY 1943 Effects of desiccation procedures on the chemical composition of feces, urine and milk. J. Lab. Clin. Med. (In press.)

WEISS, J. E., AND L. F. RETTGER 1937 The gram negative bacteriodes of the intestines. J. Bact., vol. 33, p. 423.

WERCH, S. C., R. W. JUNG, H. PLENK, A. A. DAY AND A. C. IVY 1942 Pectin and galacturonic acid and the intestinal pathogens. Am. J. Dis. Child., vol. 63, pp. 839–846.

WILLIAMS, RAY D., AND W. H. OLMSTED 1935 A biochemical method for determining indigestible residue (crude fiber) in feces: Lignin, cellulose, and non-water-soluble hemicelluloses. J. Biol. Chem., vol. 108, pp. 653–666.

WILLIAMS, RAY D., AND WILLIAM H. OLMSTED 1936 The effect of cellulose, hemicellulose and lignin on the weight of the stool: A contribution to the study of laxation in man. J. Nutrition, vol. 11, pp. 433–449.

WOODMAN, H. E., AND R. E. EVANS 1938 The mechanism of cellulose digestion in the ruminant organism. IV. Further observations from in vitro studies of the behaviour of rumen bacteria and their bearing on the problem of the nutritive value of cellulose. J. Agric. Sci., vol. 28, pp. 43–63.

THE MINIMUM DAILY REQUIREMENT OF THIAMINE OF MAN

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A portion of ingested and absorbed thiamine is metabolized in catalysis of biologic reactions for which phosphorylated thiamine (cocarboxylase) is required, a portion is stored in the tissues of the body and a portion is excreted in the urine and apparently also, as determined by Hardt and Still ('41) in the sweat. The portions excreted are lost as far as the economy of the organism is concerned.

CRITERIA FOR ESTIMATES OF REQUIREMENTS OF THIAMINE

Estimates of thiamine requirements will be influenced by the criteria which the investigator has used for judging the adequacy of the intake. If prevention of gross signs of deficiency is the criterion, the requirement will be low as compared to requirement based on the criterion of prevention of minimal abnormality. Furthermore, estimates of thiamine requirements are affected by the duration of the period of maintenance on a given, measured intake of thiamine. If the intake is just less than the minimum daily requirement and the stores of thiamine are adequate, several months may be required for demonstration of substantial depletion of tissue stores. This fact indicates the necessity of long-term studies for accurate determination of requirements of thiamine for man.

Estimates of thiamine requirement based on the content of thiamine in diets of representative groups of persons in apparent good health are subject to the criticism that the actual intakes have not been determined by analysis of the diet. Also, in such estimates standards of health have not been established, the residual effects of disease have not been known and the possible effects of deficiencies of other nutrients have not been evaluated. The nutritional status of "populations-at-hand" and "populations-at-large" is a matter of current controversy.

The data on thiamine requirements of laboratory animals cannot be dismissed as entirely inapplicable to man. The application of such data awaits a more exact knowledge of comparative rates of metabolic exchanges as well as of total metabolism, effects of synthesis of nutrients in the gastrointestinal tract of various species, variables of absorption and other factors. Recent studies by Bessey suggest that the thiamine requirement of pigeons may be higher than has been supposed and more nearly that which our studies show to be the requirement for human beings.

Complaints of ill-health are frequently the first evidence of nutritional deficiency disease in man. It is reasonable to assume that the minor deviations from the norm of physical and mental health are caused by equally minor changes of the internal milieu. Measurements of endurance and efficiency would seem to offer hope of detection of minor deviations from the norm but determinations of endurance and efficiency are exceedingly difficult to make, for they involve not only ability but also willingness to perform, and willingness is lost early in thiamine deficiency. Evidence of impairment of the feeling of well-being and of efficiency can be elicited by clinical observation. The value of such observations, however, is dependent on the degree of control of experimental conditions. It is obvious that an evaluation of the physical and psychologic status of an individual is liable to much error of judgment, particularly if the norm for the individual has not been established by close and long-continued observation.

Notwithstanding the theoretical difficulties involved, we believe that estimates of thiamine requirements can and should be based on: (1) the physical and psychologic status of the subject; (2) the condition of the tissue stores of thiamine; and (3) the biochemical status of subjects who have been maintained for several months on known intakes of thiamine. However, to obtain reliable data for evaluation of thiamine requirements it is judged necessary that the subjects of investigation be maintained continuously for several months on measured diets and in an environment which can be maintained relatively constant.

The data of earlier studies (Williams et al., '39, '40, '42, '43) and data herewith to be presented indicate that when a recent abrupt change of intake of some magnitude has not occurred, the urinary excretion of thiamine describes the intake of thiamine during and just prior to the time of test. Data on the urinary excretion of thiamine after a test dose of thiamine hydrochloride administered subcutaneously indicate that for normal man the excretion of a test dose of thiamine describes the apparent condition of the stores of thiamine. The value of the test dose in the detection of thiamine deficiency was discussed in a previous article (Mason and Williams, '42).

Since 1939 we have determined the concentration of bisulfite binding substances, pyruvic acid (a bisulfite binding substance), lactic acid and dextrose in the blood both after exercise and after administration of dextrose, levulose, sucrose, pyruvate or lactate. We have correlated these data with the clinical signs and symptoms of thiamine deficiency and also with the data on excretion of thiamine in 24 hours (hereafter called the "ordinary" excretion of thiamine) and excretion after administration of a test dose of thiamine. Our data represent observations on subjects who were known to have had adequate intakes of thiamine or to have had inadequate intakes. When the intake had been inadequate, the duration and degree of restriction also were known. Data in table 1 will serve to illustrate the relation of the clinical estimate

TABLE 1

Severe isolated restriction of thiamine (0.1 mg. per 1000 calories).

RESTRICTION OF THIAMINE	URINARY EXCRETION OF THIAMINE (MICROGRAMS)			BIOCHEMICAL STATE,[2] MG. PER 100 CC. OF BLOOD											
				Pyruvic acid				Lactic acid				Dextrose			
	Per 24 hr.	Per 4 hr. after test dose of 1 mg.	Severity of deficiency state, grade [1]	Basal state	30 min.	60 min.	120 min.	Basal state	30 min.	60 min.	120 min.	Basal state	30 min.	60 min.	120 min.
Subject 1: woman, aged 36 yr., 168 cm. tall, weighing 56.5 kg.															
days															
0	480	538	0	0.7	0.9	1.1	0.7	6.3	11.9	7.6	4.8	92	116	86	91
33	10	113	1	0.8	1.4	1.2	0.8	6.5	14.9	8.6	5.8	98	125	98	105
65	6	64	2	1.2	1.8	1.6	1.1	9.2	18.7	12.6	8.2	101	118	84	86
83	14	13	3	0.9	2.2	1.8	1.0	7.5	15.4	16.6	6.8	99	128	105	92
116	8	39	4	1.5	3.2	2 6	1.5	8.1	15.6	21.2	11.7	...	...	...	...
Subject 2: woman, aged 48 yr., 161 cm. tall, weighing 55 kg.															
0	450	548	0	0.8	1.3	1.2	0.7	3.8	7.9	6.5	3.5	91	155	100	78
35	10	96	1	1.0	1.5	1.2	1.0	6.5	9.6	8.1	6.7	100	163	98	89
63	12	74	2	1.2	2.3	1.9	0.9	7.3	19.6	16.1	5.6	97	158	96	86
83	11	58	3	1.0	2.0	2.2	1.0	6.2	16.6	15.3	7.2	102	165	138	96
110	6	37	3	1.2	2.3	2.1	1.1	5.4	17.0	14.5	6.0	105	178	125	96

[1] Clinical estimate in which 1 = minimal; 2 = moderately advanced; 3 = far advanced state of thiamine deficiency; 4 = anatomic defect (polyneuropathy).

[2] Biochemical state was determined in basal (postabsorptive) condition and for varying periods after intravenous administration of dextrose.

of the severity of thiamine deficiency to excretion of thiamine and values for pyruvic acid in the blood after administration of dextrose intravenously.

Our studies indicate in confirmation of observations of Bueding, Stein and Wortis ('41), that states of thiamine deficiency are associated with abnormally high values for pyruvic acid and lactic acid in the blood after administration of dextrose. The more elevated values are associated with the more severe states of thiamine deficiency. Determination of pyruvic acid and lactic acid in the blood after administration of dextrose intravenously or orally has proved to be the most generally applicable and reliable of the several metabolic load tests which we have used for detection of a biochemical defect produced by thiamine deficiency. Bueding and coworkers ('41) have used this type of metabolic load test for normal subjects and for those whose intake of thiamine is deficient and, together with Jolliffe (Wortis, Bueding, Stein and Jolliffe, '42), in studies of the Wernicke syndrome. It should be emphasized that we have not observed any rise of pyruvate in the blood after administration of dextrose to patients who have severe states of diabetes mellitus, except when insulin has been given just prior to, or simultaneously with, the administration of dextrose. On the other hand, we have encountered abnormally high values for pyruvic acid after administration of dextrose to patients who have mild degrees of diabetes and to other patients who have had hypermetabolism induced by feeding desiccated thyroid. We also have observed high values for pyruvic acid in patients who had hypoglycemia induced by administration of insulin. These several groups of patients were known to have received large amounts of thiamine and other vitamins of the B complex. Therefore, elevated values for pyruvic acid after administration of dextrose merely reflect a condition of excessive production or inadequate catabolism of pyruvic acid from any cause and are not pathognomonic of thiamine deficiency. However, if deficiency of other factors which have to do with metabolism of pyruvate is excluded and if other

causes for elevation of pyruvic acid in the blood can be eliminated, elevated values for pyruvic acid after administration of dextrose are significant of thiamine deficiency. Determination of lactic acid and dextrose in the blood simultaneously with the determination of pyruvic acid is helpful in interpretation of the results of the test. A return of values for pyruvic acid to normal levels after administration of thiamine hydrochloride constitutes confirmatory evidence that the biochemical defect is caused by deficiency of thiamine. However, it must be borne in mind that it may require weeks or months for complete correction of defects caused by thiamine deficiency.

SUBJECTS STUDIED

The subjects of the several studies summarized in this article were patients of the Rochester State Hospital. All of them previously had been under treatment for psychiatric abnormalities. They were recovered, however, and fully capable of exercising a decision in volunteering for this service. Possible criticism on the ground that patients recovered from mental disease might be expected to experience psychiatric disturbances from any change of environment or of diet is answered by the conditions of control. Every study involved a long period of observation first, then a long period of restriction of thiamine and a long period of observation afterward during which thiamine was administered. Only when subjects were deprived of thiamine did the abnormalities we are attributing to thiamine deficiency develop, and all subjects responded to treatment with thiamine, administered without their knowledge, by full and complete recovery from the abnormalities in question. Furthermore, these abnormalities did not develop among other groups of subjects deprived of riboflavin but not of thiamine and maintained in the same environment. Also, the observations of several investigators (Elsom et al., '40; O'Shea et al., '42; Jolliffe et al., '39) of induced thiamine deficiency in subjects who had not suffered from mental disease so closely resemble our findings as to make

it highly improbable that the responses of our subjects to restriction of thiamine were atypical.

The attention which we have given to selection and care of subjects for these studies of thiamine deficiency was considered in earlier reports. In the present article are presented data on the excretion of thiamine and the biochemical status of subjects who have been maintained on various intakes of thiamine.

The standard diets used in these experiments have been described (Williams et al., '40, '42). They were composed of foods which commonly appear on American tables and provided carbohydrate, protein and fat in conventional proportions.

METHODS OF STUDY

Thiamine deficiencies of varying degrees of severity were induced and the clinical syndrome was correlated with the apparent degree of depletion of the tissue stores and the severity of the metabolic defect. In some studies, certain phases of which have been reported elsewhere (Williams et al., '39, '40, '43), the intake of thiamine was restricted severely (0.075 or 0.10 mg. per 1000 calories), in other studies (Williams et al., '42) less severely (0.22 mg. per 1000 calories). In another investigation, part of the results of which are reported herein, the intake of thiamine was restricted only to 0.45 mg. per 1000 calories.

Determinations of thiamine. We have used the thiochrome method of Hennessy ('41) for direct determination of thiamine in foods and in urine. All quantitative data refer to thiamine hydrochloride as the standard.

Determinations of adequacy of intake and tissue stores of thiamine: test dose. For the purpose of estimating the probable condition of the tissue stores of thiamine, we administered 1.0 mg. of thiamine hydrochloride subcutaneously and determined the excretion of thiamine either for the ensuing 4 hours or for the ensuing 24 hours. The 4-hour test was performed with the subject in the basal (postabsorptive) state.

In the 24-hour tests the subject was provided with the standard diet on the day of the test. In general, the fraction of the test dose excreted in the first 4 hours after the injection approximated the fraction of the test dose recovered as extra thiamine when urine was collected for 24 hours and the usual diet was taken during the test period. Determinations of excretions of shorter duration than 4 hours are increasingly subject to errors.

Our data indicate that when the intake of thiamine on the day of test is approximately 1.0 mg., an excretion of 100 μg. or more in 24 hours represents normal ordinary excretion. Excretion of 200 μg. or more in 4 hours after subcutaneous injection of 1.0 mg. of thiamine hydrochloride, the test dose being given in the postabsorptive state, represents a satisfactory state of tissue stores of thiamine. If collection of urine after the test dose of 1.0 mg. of thiamine hydrochloride is extended to 24 hours and the subject is provided with a diet containing approximately 1.0 mg. of thiamine, excretion of 200 μg. more than the ordinary excretion of 100 μg. probably represents a satisfactory state of tissue stores.

Excretion of less than 100 μg. in 4 hours after administration of the test dose of 1.0 mg. of thiamine hydrochloride has been associated regularly with other evidence of a considerable degree of thiamine deficiency, and excretion of less than 50 μg. has been associated with evidence of severe states of thiamine deficiency.

Determination of biochemical defect: metabolic load tests. The quantities of pyruvic acid (Bueding and Wortis, '40), lactic acid (Barker and Summerson, '41) and dextrose (Miller and Van Slyke, '36) in the blood were determined after administration of dextrose intravenously or orally. The test was begun at about the twelfth basal (postabsorptive) hour with the subject at rest in bed. The sugar was administered either by vein or by mouth. For the intravenous test 0.4 gm. of dextrose (0.8 cc. of a 50%, sterile solution of dextrose) per kilogram of body weight was injected in 3 minutes. A sample of blood was taken just before and at 30, 60 and 120 minutes

after injection. The highest values both for the normal and for the thiamine deficient subject usually are obtained at 30 minutes, and this is considered the most significant value. For the oral test 50 gm. of dextrose in 250 cc. of solution was administered and 30 minutes later a second dose of 50 gm. of dextrose was given. The specimens of blood for analysis of content of pyruvic acid, lactic acid and dextrose were taken just before administration of the first dose of dextrose and at 30, 60 and 90 minutes after administration of this dose. The maximal values for pyruvic acid in this oral test were obtained in the 60- to 90-minute period; therefore, the values for pyruvic acid at 60 and 90 minutes are considered the most significant.

OBSERVATIONS

Group 1: severe restriction of thiamine (0.1 mg. to 0.175 mg. per 1000 calories). The excretion of the test dose of thiamine (a suggestive index of the condition of tissue stores of thiamine) and the level of pyruvic acid after administration of a "metabolic load" test (suggested as an index of the biochemical status) were correlated with the development of clinical signs of thiamine deficiency disease. Other details of this study will be presented in another article (Williams et al., '43).

The intake of thiamine of two physically healthy women (group 1) was restricted for 120 days to 0.1 mg. per 1000 calories. The diet was essentially that used in a previous study (Williams et al., '40). It was supplemented, per day, with 0.2 gm. of halibut liver oil fortified with irradiated ergosterol (providing 10,000 international units of vitamin A and 4000 international units of vitamin D), 80 mg. of ascorbic acid, 1.0 mg. of riboflavin, 2.0 mg. of pyridoxine, 5.0 mg. of calcium pantothenate, 20.0 mg. of nicotinamide, 0.2 gm. of inositol, 0.25 gm. of choline chloride, 0.2 gm. of ferrous sulfate and 0.6 gm. of tricalcium phosphate. The table salt was iodized. Administration of a test dose of 1 mg. of thiamine hydrochloride approximately every 2 weeks raised the average in-

TABLE 2

Group 1: severe isolated restriction of thiamine (0.1 mg. per 1000 calories).

DURATION OF INTAKE	DIET						EXCRETION OF THIAMINE (MICROGRAMS)		BIOCHEMICAL STATUS [1] MG. PER 100 CC. OF BLOOD		
	Carbohydrate	Protein	Fat	Calories	Thiamine	Ratio of thiamine to calories	Per 24 hr.	Per 4 hr. after test dose [2]	Pyruvic acid	Lactic acid	Dextrose
	Subject 1: woman, aged 36 yr., 168 cm. tall, weighing 56.5 kg. End of period of preliminary observation										
days	*gm.*	*gm.*	*gm.*		*µg.*						
10	272	71	86	2146	883	0.41	480	538	0.9	11.9	116
	Period of restriction of thiamine										
10	323	60	93	2369	233	0.10	19	200	...	...	...
30	304	58	75	2123	233	0.11	10	113	1.4	14.9	125
50	296	61	92	2256	235	0.10	6	64	...	...	...
70	298	59	99	2319	223	0.10	11	101	1.8	18.7	118
90	310	59	98	2358	231	0.10	14	13	2.2	15.4	128
110	263	52	88	2052	212	0.10	10	20	...	...	...
120	123	21	39	927	81	0.09	8	39	3.2	15.6	...
	Period of treatment with thiamine hydrochloride: 20 mg. intravenously and 60 mg. orally per day										
10	242	62	77	1909	...	...	...	...	1.5	11.0	142
30	313	56	71	2115	...	...	...	...	1.4	13.0	146
	Subject 2: woman, aged 48 yr., 161 cm. tall, weighing 55.0 kg. End of period of preliminary observation										
10	263	70	85	2097	865	0.41	450	548	1.3	7.9	155
	Period of restriction of thiamine										
10	240	64	113	2233	224	0.10	15	194	...	...	...
30	263	55	88	2064	221	0.11	10	96	1.5	9.6	163
50	285	60	89	2181	231	0.11	5	73	...	...	...
70	295	58	94	2258	221	0.10	12	74	2.3	19.6	158
90	285	54	83	2103	203	0.10	11	58	2.0	16.6	165
110	279	51	83	2067	203	0.10	8	...	2.3	17.0	178
120	89	15	26	650	64	0.10	3	37	...	...	...
	Period of treatment with thiamine hydrochloride: 15 mg. orally per day										
10	292	70	74	2114	...	...	...	...	1.6	12.6	172
30	285	62	77	2085	...	...	...	...	1.2	7.8	189

[1] 30 minutes after administration of dextrose intravenously.

[2] 1.0 mg. thiamine hydrochloride subcutaneously.

take of thiamine to 0.175 mg. per 1000 calories. Data on intake of food, intake and excretion of thiamine, excretions for 4 hours after the test dose of thiamine, and values for pyruvic acid, lactic acid and dextrose in the blood 30 minutes after intravenous administration of dextrose are contained in table 2. Approximately 30 days of treatment with thiamine were necessary to correct the biochemical defect of this severe restriction of thiamine.

Group 2: moderate, prolonged restriction of thiamine (0.22 mg. per 1000 calories). Depletion of tissue stores of thiamine and the appearance of biochemical defect were correlated with the development of clinical signs of deficiency disease. Two physically healthy subjects were maintained for 196 days on a diet which provided 0.22 mg. of thiamine per 1000 calories. The diet consisted of lean beef, cheese, milk, white bread, potatoes, sugar, canned fruits and canned vegetables as previously described (Williams et al., '42). It was supplemented with 20 gm. of autoclaved brewers' yeast. The autoclaving destroyed the thiamine in the yeast, but analysis for riboflavin and pyridoxine indicated little loss of these vitamins in autoclaving. The diet further was supplemented with 0.1 gm. of halibut liver oil fortified with irradiated ergosterol (providing 5000 international units of vitamin A and 1000 international units of vitamin D), 80 mg. of ascorbic acid, 0.2 gm. of ferrous sulfate, and 0.6 gm. of tricalcium phosphate per day. Test doses of thiamine were administered so infrequently that the intake of thiamine was not raised appreciably thereby. Clinical observations of these subjects were reported in another article (Williams et al., '42). Intake of food, intake and excretion of thiamine, excretion of thiamine for 24 hours after administration of the test dose of 1.0 mg. of thiamine hydrochloride subcutaneously and data on pyruvic acid, lactic acid and dextrose in the blood after administration of dextrose orally are presented in table 3. The biochemical defects of these two subjects developed less rapidly than those of subjects 1 and 2 (group 1) and the depletion of the stores of cocarboxylase in the tissues was less severe.

TABLE 3

Group 2: moderate restriction of thiamine (0.22 mg. per 1000 calories).

DURATION OF INTAKE	DIET						EXCRETION OF THIAMINE (MICROGRAMS)		BIOCHEMICAL STATUS [1] MG. PER 100 CC. OF BLOOD		
	Carbohydrate	Protein	Fat	Calories	Thiamine	Ratio of thiamine to calories	Per 24 hr.	Per 24 hr. after test dose [2]	Pyruvic acid	Lactic acid	Dextrose
	Subject 3: woman, aged 41 yr., 173 cm. tall, weighing 68 kg.										
	End of period of preliminary observation										
days	*gm.*	*gm.*	*gm.*		*µg.*						
10	273	60	72	1980	850	0.43	57	210	1.1	12.2	143
	Period of restriction of thiamine										
27	330	61	57	2077	460	0.22	10	145	...	...	...
60	385	63	62	2350	470	0.20	22	...	...	...	...
95	380	65	62	2338	480	0.21	18	94	1.4	13.6	142
130	392	62	81	2545	481	0.19	32	...	...	...	...
157	425	62	84	2704	489	0.18	41	...	1.7	14.3	186
194	410	63	85	2657	488	0.18	42	57	2.7	18.6	185
	Period of treatment with thiamine hydrochloride: 7.5 mg. orally per day										
134	350	60	85	2305	...	...	786	509	1.1	15.7	176
	Subject 4: woman, aged 45 yr., 166 cm. tall, weighing 54.6 kg.										
	End of period of preliminary observation										
10	260	57	70	1898	776	0.41	129	218	1.2	10.6	163
	Period of restriction of thiamine										
27	309	61	57	1993	442	0.22	19	88	1.2	8.9	178
60	308	62	58	2002	477	0.24	13	...	...	...	...
95	319	62	59	2055	467	0.23	16	93	1.2	10.4	176
130	293	54	53	1865	460	0.25	20	...	...	...	...
157	324	60	55	2031	456	0.22	21	...	2.0	14.3	170
194	281	56	56	1852	411	0.22	32	62	2.3	15.3	163
	Period of treatment with thiamine hydrochloride: 7.5 mg. orally per day										
75	271	60	56	1828	473	...	...	589	1.1	12.2	179

[1] 30 minutes after administration of dextrose intravenously.

[2] 1.0 mg. of thiamine hydrochloride subcutaneously.

Group 3: moderate restriction of thiamine (0.22 mg. per 1000 calories); inadequate provision of other vitamins of the B complex. Our earlier studies of thiamine deficiency have been criticized on the ground that provision of vitamins of the B complex other than thiamine has been too liberal and that development of thiamine deficiency may have been influenced thereby. This consideration would have a bearing on estimates of requirements for thiamine. Therefore, for group 3 which consisted of two physically healthy women thiamine was restricted to 0.22 mg. per 1000 calories, but autoclaved brewers' yeast was not provided and the only source of vitamins of the B complex was the diet. The diet otherwise was the same as that provided for the subjects of group 2. It was supplemented with 0.2 gm. of halibut liver oil fortified with irradiated ergosterol (providing 10,000 international units of vitamin A and 4000 international units of vitamin D), 80 mg. of ascorbic acid, 0.2 gm. of ferrous sulfate and 0.6 gm. of tricalcium phosphate per day. By analysis it contained 0.35 mg. of riboflavin, 1.05 mg. of pantothenic acid and (by calculation) 2.5 mg. of niacin per 1000 calories. The data obtained during 243 days of maintenance on this regimen are contained in table 4. The biochemical defect of these two subjects and the depletion of the stores of thiamine developed gradually and to about the same degree as with the subjects of group 2.

Group 4: determination of approximate thiamine requirements. Consideration of data of the previous studies made it obvious that the minimum requirement of thiamine for man was considerably more than 0.22 mg. per 1000 calories. Therefore to determine the approximate requirements six subjects were maintained on the standard diet which provided 0.22 mg. of thiamine per 1000 calories for 140 days, and additional thiamine was provided in gradually increasing amounts. In view of the possible influence of stored thiamine or cocarboxylase on the requirements, subjects with initially good stores of thiamine were compared with others known to have depleted or poor stores of this vitamin. Three subjects (subject 7, table 5 is representative of them) subsisting on this

TABLE 4

Group 3: moderate restriction of thiamine (0.22 mg. per 1000 calories without supplement of other factors of the vitamin B complex).

DURATION OF INTAKE	DIET						EXCRETION OF THIAMINE (MICROGRAMS)		BIOCHEMICAL STATUS,[1] MG. PER 100 CC. OF BLOOD		
	Carbohydrate	Protein	Fat	Calories	Thiamine	Ratio of thiamine to calories	Per 24 hr.	Per 4 hr. after test dose[2]	Pyruvic acid	Lactic acid	Dextrose
	Subject 5: woman, aged 30 yr., 152 cm. tall, weighing 45.5 kg. End of period of preliminary observation										
days	*gm.*	*gm.*	*gm.*		*µg.*						
10	238	60	100	2092	819	0.39	99	376	1.2	12.0	149
	Period of constant intake of thiamine										
27	310	70	80	2240	483	0.22	14	197	...	...	...
60	304	68	87	2271	516	0.23	8	147	1.4	12.2	146
95	312	68	73	2177	489	0.22	8	163	...	...	...
130	227	50	56	1612	334	0.21	8	...	1.9	14.8	174
157	226	51	55	1603	334	0.21	5	169	2.2	17.8	157
194	236	50	60	1684	352	0.21	4	115	2.6	18.6	128
243	231	50	58	1646	367	0.22	8	127	1.9	14.2	132
	Period of treatment with thiamine hydrochloride: 5 mg. orally per day										
10	247	50	54	1674	389	...	...	...	1.8	15.2	164
15	252	60	51	1707	365	...	...	514	1.6	12.7	105
	Subject 6: woman, aged 44 yr., 157 cm. tall, weighing 68 kg. End of period of preliminary observation										
10	240	63	105	2157	819	0.38	120	230	1.2	12.6	144
	Period of constant intake of thiamine										
27	300	70	70	2110	518	0.25	32	109	...	...	...
60	302	66	66	2066	501	0.24	9	94	1.8	16.3	168
95	231	51	53	1605	367	0.23	9	63	...	...	...
130	236	50	57	1657	334	0.20	10	...	2.0	14.8	134
157	231	51	55	1623	344	0.21	5	102	2.2	20.5	123
194	238	51	60	1696	364	0.21	7	80	2.1	13.0	145
243	236	50	58	1666	356	0.21	9	98	1.9	19.6	144
	Period of treatment with thiamine hydrochloride: 15 mg. orally per day										
10	225	45	53	1557	353	...	...	...	1.5	14.5	176
15	242	55	60	1818	368	...	...	692	1.4	12.4	124

[1] 60 minutes after administration of dextrose orally.

[2] 1.0 mg. thiamine hydrochloride subcutaneously.

TABLE 5

Group 4: determinations of approximate thiamine requirements in subjects with preliminary good stores of thiamine (subject 7) and subjects with poor stores.

DURATION OF INTAKE	DIET				INTAKE OF THIAMINE				EXCRETION OF THIAMINE (MICROGRAMS)		BIOCHEMICAL STATUS,[1] MG. PER 100 CC. OF BLOOD		
	Carbohydrate	Protein	Fat	Calories	In diet	Supplement of thiamine hydrochloride	Total	Ratio to calories	Per 24 hr.	Per 24 hr. after test dose [2]	Pyruvic acid	Lactic acid	Dextrose
Subject 7: woman, aged 33 yr., 158 cm. tall, weighing 49.6 kg.													
days	*gm.*	*gm.*	*gm.*		*µg.*	*µg.*	*µg.*						
37	333	59	55	2063	460	7500	8000	....	3610	....	1.1	10.2	195
5	356	62	58	2194	463	....	463	0.21	386	415	...	...	...
19	347	59	56	2128	460	200	660	0.31	134	310	1.3	12.4	205
20	334	60	57	2089	455	400	855	0.41	125	309	1.2	10.5	180
20	335	59	60	2215	448	600	1048	0.47	215	402	1.1	8.3	176
16	332	58	60	2100	436	800	1236	0.59	238	539	1.1	6.5	178
14	317	60	57	2021	456	1000	1456	0.72	308	560	...	...	...
14	303	58	54	1930	447	1200	1647	0.85	498	891	...	...	...
13	324	60	56	2040	462	1400	1862	0.91	547	1040	...	...	...
15	320	61	56	2028	447	1600	2047	1.0	786	1106	1.1	6.4	183
Subject 8: woman, aged 23 yr., 172 cm. tall, weighing 61 kg.													
132	397	61	83	2579	493	....	493	0.19	32	43	2.0	16.3	182
169	410	62	82	2626	470	....	470	0.19	28	61	2.4	20.5	195
19	409	62	84	2640	478	200	678	0.27	51	202	2.0	20.8	185
20	401	63	85	2621	482	400	882	0.34	88	277	1.7	16.5	188
20	400	64	84	2612	488	600	1088	0.42	136	371	1.2	14.2	172
16	396	62	88	2624	480	800	1280	0.49	202	431	1.2	16.9	178
14	368	62	85	2485	456	1000	1456	0.59	287	520	...	...	...
14	365	63	86	2486	497	1200	1697	0.68	557	899	...	...	...
13	360	63	72	2340	492	1400	1892	0.81	595	965	...	...	...
15	355	63	61	2221	479	1600	2079	0.94	731	1189	1.1	7.8	164

[1] 30 minutes after administration of dextrose intravenously.

[2] 1.0 mg. thiamine hydrochloride subcutaneously.

standard diet were given 7.5 mg. of thiamine hydrochloride per day for 37 days. They thus had opportunity to accumulate stores of thiamine. Three other subjects (subject 8, table 5 is representative of them) had been maintained for 169 days on the same diet without supplementary thiamine. Their stores of thiamine undoubtedly were depleted. During the study of requirements the diet was supplemented, per day, with 20 gm. of autoclaved brewers' yeast, 0.1 gm. of halibut liver oil fortified with irradiated ergosterol (providing 5000 international units of vitamin A and 1000 international units of vitamin D), 80 mg. of ascorbic acid, 0.2 gm. of ferrous sulfate and 0.6 gm. of tricalcium phosphate. The table salt was iodized. A report of clinical observations of these subjects has been made (Williams et al., '42). Data on intake of food, intake and excretion of thiamine, excretions for 24 hours after test doses of 1 mg. of thiamine hydrochloride subcutaneously and values for pyruvic acid, lactic acid and dextrose in the blood after administration of dextrose intravenously are presented in table 5. Evidence of inadequate intake of thiamine was not observed in the subjects who initially had good stores of thiamine so long as the intake exceeded 0.4 mg. per 1000 calories. However, there was some evidence of inadequate intake of thiamine in subjects who initially had poor stores of thiamine so long as the intake was less than 0.5 mg. per 1000 calories. Therefore, it appeared that an intake of approximately 0.45 mg. of thiamine per 1000 calories should be regarded as a minimal intake for these subjects initially possessing reasonably good stores of thiamine.

Groups 5 and 6: intake of thiamine approximating the minimum requirement. In another study, not yet reported, five women were maintained on a standard diet which provided 0.45 mg. of thiamine per 1000 calories for a period of 243 days. The diet, composed principally of lean beef, butter, heavy cream, hydrogenated vegetable fat, bread, sugar, potatoes, canned fruits and canned vegetables, was identical in every respect to the diet prescribed for group 3 with the exception that the bread component of the diet was made of a whole grain wheat flour (group 5, subjects 9 and 10, table 6),

TABLE 6

Group 5: subjects receiving "minimum" daily requirement of thiamine (0.45 mg. per 1000 calories).

DURATION OF INTAKE	DIET						EXCRETION OF THIAMINE (MICROGRAMS)		BIOCHEMICAL STATUS,[1] MG. PER 100 CC. OF BLOOD		
	Carbohydrate	Protein	Fat	Calories	Thiamine	Ratio of thiamine to calories	Per 24 hr.	Per 4 hr. after test dose[2]	Pyruvic acid	Lactic acid	Dextrose
	Subject 9: woman, aged 34 yr., 158 cm. tall, weighing 51.4 kg. End of period of preliminary observation										
days	*gm.*	*gm.*	*gm.*		*mg.*						
10	327	68	83	2327	843	0.36	215	215	1.1	7.8	166
	Period of constant intake of "minimal" requirement of thiamine										
27	307	66	87	2275	1046	0.45	124	198	...	...	...
60	328	69	88	2380	1082	0.45	108	139	1.2	8.9	167
95	341	68	74	2302	1054	0.46	80	162	...	...	...
130	273	58	63	1891	796	0.42	52	...	1.7	12.4	168
157	304	71	70	2130	920	0.43	43	199	1.5	10.3	143
194	320	88	80	2352	1035	0.44	46	195	1.5	10.1	156
243	324	66	80	2280	1030	0.45	83	162	1.7	14.9	118
	Period of treatment with thiamine hydrochloride: 15 mg. orally per day										
7	342	65	74	2294	1081	...	...	...	1.7	15.7	128
15	330	67	76	2272	1052	...	...	586	1.4	13.3	120
	Subject 10: woman, aged 21 yr., 154 cm. tall, weighing 59.6 kg. End of period of preliminary observation										
10	276	63	105	2301	819	0.36	255	232	1.1	8.4	165
	Period of constant intake of "minimal" requirement of thiamine										
27	328	65	89	2373	1044	0.44	162	225	...	...	...
60	344	69	87	2435	1095	0.45	164	202	1.2	6.9	170
95	344	69	75	2327	1069	0.46	166	215	...	...	...
130	420	84	96	2880	1305	0.45	212	...	1.2	8.6	176
157	422	85	95	2883	1338	0.43	213	234	1.2	8.2	191
194	440	84	102	3014	1351	0.45	225	239	1.1	5.5	197
243	335	68	80	2332	1054	0.45	174	235	1.1	6.3	190
	Period of treatment with thiamine hydrochloride: 15 mg. orally per day										
7	373	65	74	2458	1081	...	...	...	1.2	8.3	140
15	368	64	70	2358	1061	...	...	552	1.2	10.3	149

[1] 60 minutes after administration of dextrose orally.

[2] 1.0 mg. thiamine hydrochloride subcutaneously.

TABLE 7

Group 6: other subjects receiving "minimum" daily requirement of thiamine (0.45 mg. per 1000 calories).

DURATION OF INTAKE	DIET						EXCRETION OF THIAMINE (MICROGRAMS)		BIOCHEMICAL STATUS,[1] MG. PER 100 CC. OF BLOOD		
	Carbohydrate	Protein	Fat	Calories	Thiamine	Ratio of thiamine to calories	Per 24 hr.	Per 4 hr. after test dose[2]	Pyruvic acid	Lactic acid	Dextrose
	Subject 11: woman, aged 37 yr., 157 cm. tall, weighing 74.6 kg.										
	End of period of preliminary observation										
days	*gm.*	*gm.*	*gm.*		*µg.*						
10	250	60	100	2140	850	0.40	369	282	1.2	10.2	168
	Period of constant intake of "minimal" requirement of thiamine										
27	350	70	58	2202	1034	0.47	240	206	...	...	...
60	337	67	67	2219	1065	0.48	106	166	1.3	8.3	165
95	354	67	72	2332	1062	0.46	67	138	...	...	...
130	353	65	75	2347	1006	0.43	81	...	1.7	14.4	155
157	349	65	75	2331	1012	0.43	64	88	1.7	11.8	178
194	359	68	79	2419	1034	0.43	43	156	1.8	13.8	175
243	347	68	79	2371	1067	0.45	84	119	1.9	15.7	141
	Period of treatment with thiamine hydrochloride: 15 mg. orally per day										
7	348	64	72	2296	1081	...	...	...	1.6	15.3	124
15	340	65	71	2259	1079	...	...	670	1.5	16.4	161
	Subject 12: woman, aged 42 yr., 173 cm. tall, weighing 66.0 kg.										
	End of period of preliminary observation										
10	309	69	83	2259	843	0.37	227	256	1.2	7.6	174
	Period of constant intake of "minimal" requirement of thiamine										
27	330	70	77	2293	1035	0.45	114	190	...	...	...
60	292	60	79	2119	856	0.40	144	244	1.3	7.4	167
95	314	63	68	2120	973	0.46	119	168	...	...	...
130	323	68	75	2239	1010	0.45	153	...	1.4	10.6	174
157	333	66	74	2262	1011	0.45	115	196	1.5	12.1	182
194	340	68	80	2352	1050	0.45	119	167	1.3	8.7	153
243	315	68	78	2234	1050	0.47	146	235	1.9	11.4	185
	Period of treatment with thiamine hydrochloride: 15 mg. orally per day										
7	357	64	72	2332	1081	...	....	...	1.5	14.0	133
15	340	65	70	2250	1065	...	....	953	1.4	14.6	173
	Subject 13: woman, aged 27 yr., 159 cm. tall, weighing 68.2 kg.										
	End of period of preliminary observation										
10	306	65	84	2240	819	0.37	146	279	1.2	8.4	167
	Period of constant intake of "minimal" requirement of thiamine										
27	345	66	76	2328	1042	0.45	62	244	...	...	...
60	343	67	67	2243	1092	0.49	80	331	1.2	8.6	168
95	324	68	73	2225	1064	0.48	73	179	...	...	...
130	313	66	75	2191	1003	0.46	82	156	1.5	14.1	125
157	308	67	74	2166	1024	0.47	93	206	1.6	13.3	111
194	322	66	80	2272	1040	0.46	84	168	1.8	8.3	148
243	300	67	77	2161	1053	0.49	108	194	1.6	7.7	150
	Period of treatment with thiamine hydrochloride: 15 mg. orally per day										
7	353	64	72	2316	1081	...	...	...	1.5	12.8	138
15	340	65	70	2250	1045	...	...	437	1.4	11.6	138

[1] 60 minutes after administration of dextrose orally.

[2] 1.0 mg. of thiamine hydrochloride subcutaneously.

or of a flour (group 6, subjects 11, 12 and 13, table 7) to which thiamine (2.5 mg. per pound of flour), niacin (18.0 mg. per pound of flour) and nonfat milk solids (six parts per 100 parts of flour) had been added. The diet provided 0.45 mg. of thiamine and 0.45 mg. of riboflavin per 1000 calories. Again we were mindful of the criticism that too liberal provision of nutrients other than thiamine might influence development of thiamine deficiency, and, therefore, the diet was supplemented only with 0.2 gm. of halibut liver oil fortified with irradiated ergosterol (providing 10,000 international units of vitamin A and 4000 international units of vitamin D), 80 mg. of ascorbic acid, 0.2 gm. of ferrous sulfate and 0.6 gm. of tricalcium phosphate. Thus, the only source of the vitamins of the B complex was the food of the diet.

Data on intake of food, intake and excretion of thiamine, excretion after a test dose of thiamine in 4 hours and values for pyruvic acid, lactic acid and dextrose in the blood after administration of dextrose orally are presented in table 6 for subjects 9 and 10 (subjects receiving a diet in which bread component was made of whole grain wheat flour) and in table 7 for subjects 11, 12 and 13 (subjects receiving diet in which bread component was made of "enriched" flour). Subject 9 of group 5 developed a biochemical defect with evidence of depleted stores of thiamine; subject 10 did not (table 6). Subjects 11 and 13 of group 6 developed a biochemical defect and evidence of depleted stores of thiamine, whereas subject 12 showed some but little change (table 7).

COMMENT

Elsom and co-workers ('42), in a study of nine subjects who received from 204 to 773 μg. of thiamine per day (0.12 to 0.40 mg. per 1000 calories), reached the conclusion that the minimum absolute thiamine intake compatible with health was about 651 μg. per day, or a thiamine to calorie ratio of about 0.35 (0.35 mg. per 1000 calories). The three subjects of her study on the higher intakes of thiamine (0.35 to 0.40 mg. per 1000 calories) were maintained on the regimen for

periods of 28 to 36 days. These periods, in our opinion, are not long enough for appreciable change in stores of thiamine. No data were presented which would describe the stores of thiamine or the biochemical status of the subjects studied and for these reasons we are inclined to believe that the minimum requirement of thiamine will be found higher than 0.35 mg. per 1000 calories.

We also are inclined to believe that Melnick ('42), basing his conclusions primarily on excretions of thiamine, has set too low a value for the minimum requirement of thiamine. From thiamine balance studies he estimated the thiamine requirement of the adult to be 0.35 mg. per 1000 calories and recommended a daily intake of 0.5 mg. per 1000 calories.

The minimum requirement of thiamine should be defined, in our opinion, as the smallest amount of this vitamin which will make for performance of tissue respiration at normal rates and under conditions of normal concentrations of intermediary metabolites. If the intake of thiamine is less than such a minimum amount, tissue respiration can continue under normal conditions only so long as stores of thiamine in the tissues are not severely depleted. It also is reasonable to assume that the biochemical reactions catalyzed by cocarboxylase proceed more slowly when the stores of thiamine have become depleted in any degree, and therefore that depletion of these stores in itself represents an abnormality. In that case the minimum requirement should be sufficient to prevent such depletion. However, it is also possible that increased concentrations of intermediary metabolites in blood and tissues might exert a mass action effect of a degree which would enable the tissues to respire at normal rates even in the absence of a normal tissue complement of thiamine. Elevation of the concentrations of pyruvic acid and of other intermediary metabolites in the blood is regularly encountered in thiamine deficiency. This may reflect an attempt on the part of the organism to use the available supply of thiamine as effectively as possible through a mass action of increased levels of intermediary metabolites. A lowering of basal

metabolic rate is irregularly encountered in thiamine deficiency. This effect of thiamine depletion also may reflect an attempt on the part of the organism to conserve its stores of thiamine.

These considerations suggest two distinct requirements of thiamine: (1) for tissue respiration under conditions of normal concentrations of intermediary metabolites and normal metabolic rates and, (2) for tissue respiration under conditions of elevated levels of intermediary metabolites and lowered rates. The former might be called a normal minimum requirement, and the latter an abnormal minimum requirement for thiamine.

Requirement of insulin for the diabetic patient is analogous. Sixty units of insulin per day may be required for maintenance of normal concentrations of the dextrose and other intermediary metabolites of a given diabetic patient, and yet 20 units of insulin per day may be sufficient to prevent dangerous elevation of dextrose and other metabolites in the blood and tissues. In the latter instance increased concentrations of intermediary metabolites have reduced the absolute requirement for insulin, possibly through effects of mass action. In other words, 60 units of insulin may be regarded as the normal minimum requirement for insulin in the example cited and 20 units the abnormal minimum requirement. Deleterious effects of maintenance of a diabetic patient on an abnormal minimum requirement of insulin may fail to become apparent for several months or years.

The requirement of thyroxine for the myxedematous patient is another analogy. The amount of desiccated thyroid gland required for maintenance of the basal metabolic rate at −20% is usually less than half that required for maintenance of the metabolic rate at a normal level. The organism becomes adjusted to the somewhat low metabolic rate, not, however, with complete immunity. Deleterious effects of maintenance on the less-than-normal minimum requirement of the thyroid hormone make themselves apparent after many months or years.

The organism has a considerable ability of adaptation to abnormal internal environmental conditions, but if adaptation is forced for long periods of time, deleterious effects may be anticipated. Meyers ('41) has adduced evidence of adaptation to chronic low intakes of thiamine in studies of healthy Javanese laborers. He interprets these data as indicating that the organism can make a satisfactory adjustment to a probable thiamine intake of 0.6 to 1.0 mg. per day. His estimates of status of thiamine nutrition were based not only on ordinary excretions of thiamine but also on excretions after administration of test doses of thiamine, and if his method of estimating thiamine (thiochrome method of Westenbrink and Goudsmit) is comparable to the one we have used (thiochrome method of Hennessy), his data on excretions would seem to indicate that the intake of thiamine had been approximately in the range of intake which he estimated and that stores of thiamine were low. The biochemical status of the healthy Javanese laborers was not determined, and the criteria for determining their state of health were not mentioned. The thiamine content of the diets prior to the period of test was not known. The report contains the comment that beriberi appears regularly in the Netherlands East Indies although not everywhere with equally frequent distribution. It might be assumed, therefore, that adaptation to low intake of thiamine was not equally effective in all of the natives.

We have encountered evidence that some subjects require less thiamine than others of the same age, habits and apparent physical status. We also have observed that shifting from a high level of intake of thiamine to a low level has been associated almost always with mild complaints of fatigue and anorexia which disappeared within a few days; whereas shifting from a moderate level of intake to an equally low level has not been associated with any complaints until actual deficiency of thiamine occurred.

The data on subjects of group 1, who had been maintained on the diet which provided 0.1 mg. of thiamine per 1000 calories, and who had been given the test dose of thiamine

hydrochloride every 2 weeks (subjects 1 and 2, table 2), indicate that with the intake at this level the ordinary excretions of thiamine decreased rapidly (reflecting the current intake of thiamine). The amounts of thiamine excreted after the successive test dose of thiamine also decreased rapidly and progressively (reflecting rapid depletion of the stores of thiamine in the tissues). Stepwise with the decrease of excretion of thiamine after the test dose, the values for pyruvic acid after administration of dextrose intravenously became progressively higher. That the biochemical defect was caused by deficiency of thiamine is indicated by the facts that (1) nutrients other than thiamine had been supplied adequately, and (2) the biochemical defect disappeared when thiamine was again provided and no other change was made in the regimen. It was the data of this study that led to the conclusion that determination of excretion of thiamine in the 4 hours after administration of a test dose was a valid procedure for estimation of the condition of the tissue stores of thiamine, and that, in the absence of organic disease or of deficiency of other nutrients, determination of pyruvic acid after administration of a metabolic load of dextrose is a useful technic for detection of thiamine deficiency.

Data for groups 2 and 3 on excretion of the test dose of thiamine indicate that an intake of 0.22 mg. of thiamine per 1000 calories was associated with gradual depletion of tissue stores of thiamine and a gradual development of a biochemical defect. Subjects 3 and 4 (group 2) who had received supplements of autoclaved brewers' yeast had rather severe states of thiamine deficiency at the end of 196 days. Subjects who did not receive supplements of autoclaved brewers' yeast (group 3, table 4) were relatively less severely affected by restriction of thiamine to 0.22 mg. per 1000 calories for a period of 243 days. However, there were two fundamental differences in the regimens of groups 2 and 3. The intake of food of subjects of group 2 was usually more than 2000 calories per day, but the intake of food of subjects of group 3 was, with the exception of the first 60 to 100 days, less than 1700

calories per day. However, the ratio of thiamine to calories for both groups 2 and 3 was approximately the same, 0.18 to 0.23. A more important difference in the regimens of the two groups of subjects was that during the period of restriction of thiamine subjects of group 2 received only two injections of 1.0 mg. of thiamine (test dose) whereas the subjects of group 3 received five injections of the test dose of thiamine. The periodic administration of a small dose of thiamine exerted a recuperative effect which was clinically evident for 1 to 2 weeks after each injection.

It may be significant that the subjects who did not receive a supplement of autoclaved brewers' yeast tended to gain weight. To prevent gain their caloric intake was reduced by a fourth. The reduction of intake was accomplished without alteration of the ratios of carbohydrate, protein and fat.

A comparison of data concerning subjects (group 4) with initially good stores of thiamine (subject 7, table 5) with data concerning other subjects with initially poor stores of thiamine (subject 8, table 5) in the study of minimum daily requirements of thiamine indicated that for subjects with initially good stores of thiamine an intake of 0.4 mg. per 1000 calories would be required, and that for subjects with initially poor stores 0.5 mg. per 1000 calories would be required for prevention of evidence of biochemical abnormality. The duration of each intake was relatively short (14 to 20 days) so that these data alone cannot be accepted as conclusive evidence of requirement; however, they do indicate that for subjects who have ordinary stores of thiamine an intake of about 0.45 mg. per 1000 calories is sufficient to prevent the development of thiamine deficiency for short periods of time.

The hypothesis that an intake level of 0.45 mg. per 1000 calories is insufficient to assure prevention of serious depletion of tissue stores of thiamine over longer periods is borne out by the fact that of the five subjects of groups 5 and 6 who received 0.45 mg. of thiamine per 1000 calories three subjects (subject 9, table 6; subjects 11 and 13, table 7) consistently excreted less than 100 μg. of thiamine per day, and subjects 9

and 11 consistently excreted less than 200 μg. of thiamine in 4 hours after test doses of 1 mg. of thiamine hydrochloride. Also, there was some degree of increase in values for pyruvic acid after administration of dextrose in all of these subjects except subject 10 (table 6). There is some suggestion that this young, robust subject utilized thiamine more efficiently than the others; however, a greater storage of thiamine at the beginning of the study or a lower requirement for this substance might account for her better nutritional status.

The diet of the subjects in groups 5 and 6 was low in content of riboflavin and pantothenic acid by analysis, and in niacin by calculation. That deficiency of thiamine was probably the principal cause of elevation of values for pyruvic acid after administration of dextrose, is indicated by the return of the values to lower levels when thiamine was again provided. In studies of isolated riboflavin deficiency and of isolated niacin deficiency elevation of values for pyruvic acid after administration of dextrose has not been observed.

SUMMARY AND CONCLUSIONS

Determination of excretion of thiamine after administration of a test dose of 1.0 mg. of thiamine hydrochloride subcutaneously appears to be a valid test for assessment of the condition of tissue stores of thiamine and, respectively, of cocarboxylase. Determination of pyruvic acid in the blood after administration of dextrose intravenously or orally appears to be a valid test for detection of a biochemical defect of thiamine deficiency, provided that other causes of abnormal elevation can be excluded. Abnormal elevation of values for pyruvic acid in the blood after administration of dextrose was observed under various abnormal conditions. No rise was observed in cases of severe diabetes mellitus when insulin was not given. However, a rise was noted when insulin was provided just before or at the time of the administration of the dextrose. Also, in states of mild diabetes, in hypoglycemia induced by administration of insulin and in hypermetabolism,

abnormally high values for pyruvic acid in the blood after administration of dextrose have been encountered.

Restriction of the thiamine intake of two subjects to from 0.1 to 0.175 mg. per 1000 calories was associated with rapid depletion of the tissue stores of thiamine and early development of a biochemical defect.

Restriction of the thiamine intake of four subjects to 0.22 mg. per 1000 calories was associated with a slow depletion of the tissue stores of thiamine and slow development of the biochemical defect.

An intake of 0.45 mg. per 1000 calories was associated in three of five subjects with a slight degree of depletion of tissue stores of cocarboxylase, and in four of the five with slow development of a mild degree of biochemical defect. Although the disturbance of metabolism at this level of intake of thiamine was slight, such an intake (0.45 mg. of thiamine per 1000 calories) cannot be regarded as representing more than the minimum daily requirement of thiamine for these five subjects who subsisted on a diet to which carbohydrate, protein and fat contributed in proportions conventional for the majority of American and European diets.

In the light of these findings, and to provide some safeguard against higher proportions of nonfat calories in some diets, individual variability and other causes of increased minimum requirement, the daily allowance of 0.6 mg. of thiamine per 1000 calories, recommended by the Food and Nutrition Board of the National Research Council, is certainly none too high.

LITERATURE CITED

BARKER, S. B., AND W. H. SUMMERSON 1941 The colorimetric determination of lactic acid in biological material. J. Biol. Chem., vol. 138, pp. 535–554.

BESSEY, P. A. Personal communication to the authors.

BUEDING, ERNEST, AND HERMAN WORTIS 1940 The stabilization and determination of pyruvic acid in the blood. J. Biol. Chem., vol. 133, pp. 585–591.

BUEDING, ERNEST, M. H. STEIN AND HERMAN WORTIS 1941 Blood pyruvate curves following glucose ingestion in normal and thiamine-deficient subjects. J. Biol. Chem., vol. 140, pp. 697–703.

ELSOM, K. O., F. H. LEWY AND G. W. HEUBLEIN 1940 Clinical studies of experimental human vitamin B complex deficiency. Am. J. Med. Sci., vol. 200, pp. 757–764.

ELSOM, K. O., J. G. REINHOLD, J. T. L. NICHOLSON AND CHARLOTTE CHORNOCK 1942 Studies of the B vitamins in human subjects. V. The normal requirement for thiamine; some factors influencing its utilization and excretion. Am. J. Med. Sci., vol. 203, pp. 569–577.

HARDT, L. L., AND E. U. STILL 1941 Thiamine in sweat. Proc. Soc. Exper. Biol. Med., vol. 48, pp. 704–707.

HENNESSY, D. J. 1941 Chemical methods for determination of vitamin B_1. Indust. and Eng. Chem. (Analyt. Ed.), vol. 13, pp. 216–218.

JOLLIFFE, NORMAN, ROBERT GOODHART, JOSEPH GENNIS AND J. K. CLINE 1939 The experimental production of vitamin B_1 deficiency in normal subjects. The dependence of the urinary excretion of thiamin on the dietary intake of vitamin B_1. Am. J. Med. Sci., vol. 198, pp. 198–211.

MASON, H. L., AND R. D. WILLIAMS 1942 The urinary excretion of thiamine as an index of the nutritional level: assessment of the value of a test dose. J. Clin. Invest., vol. 21, pp. 247–255.

MELNICK, DANIEL 1942 Vitamin B_1 (thiamine) requirements of man. Presented in the Symposium on "Human Requirements for the Vitamins." Annual meeting of the American Institute of Nutrition, Boston, Massachusetts, April 1.

MEYERS, F. M. 1941 Possible adaptation to a low vitamin B_1 intake. Am. J. Med. Sci., vol. 201, pp. 785–789.

MILLER, B. F., AND D. D. VAN SLYKE 1936 A direct microtitration method for blood sugar. J. Biol. Chem., vol. 114, pp. 583–595.

O'SHEA, HARRIET E., K. O. ELSOM AND RUTH V. HIGBE 1942 Studies of the B vitamins in the human subject. IV. Mental changes in experimental deficiency. Am. J. Med. Sci., vol. 203, pp. 388–397.

WILLIAMS, R. D., H. L. MASON AND B. F. SMITH 1939 Induced vitamin B_1 deficiency in human subjects. Proc. Staff Meet., Mayo Clin., vol. 14, pp. 787–793.

WILLIAMS, R. D., H. L. MASON, M. H. POWER AND R. M. WILDER 1943 Observations on induced thiamine deficiency in man: relation of depletion of thiamine to development of biochemical defect and of polyneuropathy. Arch. Int. Med. (In press.)

WILLIAMS, R. D., H. L. MASON, B. F. SMITH AND R. M. WILDER 1942 Induced thiamine (vitamin B_1) deficiency and the thiamine requirement of man: further observations. Arch. Int. Med, vol. 69, pp. 721–738.

WILLIAMS, R. D., H. L. MASON, R. M. WILDER AND B. F. SMITH 1940 Observations on induced thiamine (vitamin B_1) deficiency in man. Arch. Int. Med., vol. 66, pp. 785–799.

WORTIS, HERMAN, ERNEST BUEDING, M. H. STEIN AND NORMAN JOLLIFFE 1942 Pyruvic acid studies in the Wernicke syndrome. Arch. Neurol. and Psychiat., vol. 47, pp. 215–222.

CUCURBIT SEED GLOBULINS

II. USE AS SUBSTITUTES FOR EDESTIN IN EXPERIMENTAL DIETS

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A preliminary report has recently been made of the nutritive value of the globulins prepared from a variety of both Cucurbita moschata and Citrullus vulgaris in comparison with edestin from hemp seed and with lactalbumin and casein (Vickery, Smith, Hubbell and Nolan, '41). Inasmuch as further work of a similar nature is not at present contemplated, feeding experiments with globulins of three additional varieties of cucurbit seeds are here reported.

Globulins from seeds of Citrullus vulgaris, var. Phinney's early watermelon, of Cucumis sativus, var. Woodruff hybrid cucumber, and of Cucurbita pepo, var. early white bush scallop, were prepared by the method previously described.

Male albino rats of the Connecticut Agricultural Experiment Station strain were weaned at 21 days of age, with an average body weight of 48 gm., and were fed diets of the following percentage composition: protein 20, butterfat 9, lard 20, starch 49, salts 351 (Hubbell, Mendel and Wakeman, '37) 2; 10 drops of cod liver oil were supplied as a daily supplement, and the amount of yeast given varied with the weight of the animal (400 mg. daily at the start, 600 mg. from 100 to 150 gm., and 800 mg. from 150 to 200 gm. of body weight, when the tests were terminated). Table 1 shows the results of both groups of feeding tests.

The average daily gains of the rats supplied globulins varied from 2.8 gm., with the globulin of Cucurbita moschata, to 3.3 gm. with edestin. For the controls fed casein or lactalbumin, the rate of growth was considerably higher, that is, from 4.2 gm. to 4.7 gm. for the period of active growth studied. It is evident that, under the conditions of this experiment, the

TABLE 1

Comparative rates of growth of rats furnished seed globulins, casein, or lactalbumin.

PROTEIN	NO. OF ANIMALS	AVERAGE DAILY GAIN FROM 60 TO 200 GM.	AVERAGE FOOD INTAKE PER GM. OF WEIGHT GAINED
		gm.	*gm.*
Casein (1941)	10	4.2	2.4
(1942)	14	4.5	2.3
Lactalbumin (1941)	7	4.7	2.2
(1942)	8	4.4	2.2
Edestin (1941)	8	3.3	2.7
Cucurbit globulins			
Cucurbita moschata, var. striped cushaw squash (1941)	8	3.2	2.7
(1942)	8	2.8	3.0
Cucurbita pepo, var. early white bush scallop (1942)	7	3.0	3.0
Citrullus vulgaris, var. Halbert honey watermelon (1941)	5	3.2	2.7
Citrullus vulgaris, var. Phinney's early watermelon (1942)	7	3.0	2.8
Cucumis sativus, var. Woodruff hybrid cucumber (1942)	6	3.1	3.0

six vegetable globulins are essentially equivalent in nutritive value as measured by the growth of the young rat, and are all inferior to casein and to lactalbumin. This conclusion is supported by the larger food intake of the rats to which the vegetable globulins were administered. For each gram of weight increase, these animals consumed about 25% more food than the rats supplied with casein or lactalbumin.

As was indicated in the previous report, diarrhea was a complicating factor in the growth of many of the rats fed the vegetable globulins. In the earlier experiments (marked 1941 in the table) this was more pronounced with watermelon seed globulin than with the globulin from the seed of Cucurbita moschata. In the present series, however, there was very little difference between the two globulins in this respect, and the same difficulty was also experienced with the two new varieties. The decreased rate of growth of the rats fed globulin from Cucurbita moschata in the experiments of 1942 seemed to be associated with the increased diarrhea, but the small number of animals available for observation made it impossible to be certain of this point.

SUMMARY

Globulins from the seeds of two varieties of Citrullus vulgaris and one variety each of Cucurbita moschata, Cucurbita pepo, and Cucumis sativus have been found to be satisfactory substitutes for edestin for the growth of the albino rat from weaning to a body weight of 200 gm.

LITERATURE CITED

HUBBELL, R. B., L. B. MENDEL AND A. J. WAKEMAN 1937 A new salt mixture for use in experimental diets. J. Nutrition, vol. 14, p. 273.

VICKERY, H. B., E. L. SMITH, R. B. HUBBELL AND L. S. NOLAN 1941 Cucurbit seed globulins. I. Amino acid composition and preliminary tests of nutritive value. J. Biol. Chem., vol. 140, p. 613.